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RECENT PROGRESS IN IMMUNOLOGY OF SCHISTOSOMIASIS

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Immunological research in schistosomiasis has made important progress during the last years. Two major reasons explain the present interest in a field that was investigated before only by a few laboratories.

On the one hand, there are 350 million people in the world infected by schistosomes, with a large impact on public health and economical development in the countries where this severe parasitic disease occurs.

On the other hand, presently available therapeutics, despite their efficiency, cannot prevent infection and therefore have little effect on the level of endemicity.

Prophylactic methods are either too geographically limited or have so severe consequence on environment that large scale use is prohibited.

Thus, it was logical to think that immunological control of schistosomiasis might be of value in the fight against this parasitic disease.

But it has become evident during the past few years that some other considerations motivated the interest in the immunological study of schistosomiasis. As discussed later, experimental schistosomiasis is a particularly rich model for investigating many fundamental immunological mechanisms, such as antigenic mimicry, some particular effector mechan-

isms, immunosuppression or regulatory influence of circulating antigens or soluble immune complexes.

Even if it is not certain that schistosomiasis will take benefit from the immunological studies, the theoretical interest is so great that immunology has already and will continue to take more and more advantage of the study of schistosomes.

The above-mentioned reasons probably explain why, recently important progress has been made both in applied immunology, in fundamental immunology such as immunodiagnosis, or in findings on effector immune mechanisms or mechanisms of survival of the parasite.

According to the main experimental models, particularly in rodents, immune response against schistosomes appears to result from three prominent features :

— Antigenicity of schistosomes ;

— Effector mechanisms ;

— Mechanisms of survival in the immune host.

These three topics have been intensively investigated during the last few years and encompass some of the more important progresses made in this field.

Schistosome Antigens

The complex antigenic structure of schistosomes is a fact now clearly estab-

lished. Modern methods of analysis, for example, bidimensional electrophoresis, demonstrate at least 60 different antigens. But the main feature is the existence, among this mosaïc of antigens, of highly specific antigens which are also strong immunogens. Some of them are genus specific, others species or stage specific.

Affinity chromatography using either enzymatic inhibitors or immunosorbents has recently permitted the purification of some of these specific antigens.

Most of them carry some important enzymatic activities while others elicit preferential production of some immunoglobulin classes, such as IgE. The immunochemical study of schistosome antigens however has its particular interest in the correlative investigation of their immunogenicity and their origin.

It has become evident now that parasitic infection by schistosomes not only consists in the presence of parasites in the bloodstream but also in the existence of circulating soluble antigens in blood and in urine as well. The numerous circulating antigens may originate from the parasitic membrane (the turn over of which is important), from metabolic products of the digestive tract, from excretion-secretion products and also from somatic antigens released by the worm either during immunological rejection or efficient treatment. Accordingly, various antigens have been demonstrated as circulating antigens, in experimental as well as in human schistosomiasis, and subsequently soluble immune complexes were also characterized.

Progress in the study of schistosome antigens allowed (1) for purified antigens to be obtained, the practical interest of which is evident, and (2) for some antigens to be characterized, particularly the circulating ones, which play an important role in the regulation of the immune response.

For many years, it was not certain that a precise knowledge of schistosome antigens might lead to anything else than an improvement in the serology of schistosomiasis. But the evidence now obtained suggests that some protective antigens do exist in the parasite. It is not too optimistic to think that isolation of such antigens combined with the use of adequate adjuvants might offer the possibility of vaccination in schistosomiasis.

Effector Mechanisms

From a general point of view, parasitic infection, by protozoas as well as by helminths, induces antibody production and in some cases cell mediated immunity.

Analysis of the various humoral and cellular factors during the course of human or experimental schistosomiasis leads one to consider that at least *in vitro,* effector mechanisms are the result of cooperation or association of antibody and unsensitized cells.

Many mechanisms have been described during the last years, which are summarized in Table 1. It can be seen that *in vitro* cytotoxicity against schistosomules is the consequence of association of IgG or IgE antibodies with various normal cells : polymorphonuclears, neutrophils, eosinophils or macrophages.

TABLE 1. *In vitro* Effector Mechanisms

Mechanisms	Ig classes	Complement	Hosts
Serotoxicity:			
Cytotoxic antibody	IgG	C+	Rhesus monkey, Man, Rat, Rabbit, Guinea pig
Cell dependent cytotoxicity :			
Antibody + PMN leucocytes	IgG	C+	Rat
Antibody + neutrophils . .	IgG$_2$	C+	Guinea pig
Antibody + eosinophils . .	IgG$_1$	C—	Man, Baboons, Mice
Heat labile Antibody + Macrophages	IgE	C—	Rat
Heat stable Antibody + Macrophages	IgG$_1$ (?)	C—	Rat

Some of these mechanisms are particular to parasites. In this respect, intervention of eosinophils, or IgE antibody must be pointed out. The role of complement is also demonstrated.

Two important remarks should be made about these effector mechanisms. First, it is important to mention that any experiment involving sensitized cells from immune animals has failed, though cellular immunity has been clearly demonstrated in some hosts like, for example, the rat. Then, most of the data were obtained *in vitro* and until now no evidence has been provided, that these mechanisms are efficient *in vivo*. Recent experiments however suggest a correlation between the *in vitro* role of eosinophils and the *in vivo* activity of anti-eosinophil sera on induction of immunity.

Survival Mechanism of Schistosomes

The relative ineffectiveness of the immune response induced by parasites, the consequences of which is the long survival in the immune host, has been extensively investigated during the last years. Some of the most important and recently established mechanisms will be discussed here.

1) *Immunosuppression*

Many mechanisms of immunosuppression may be induced by schistosomes. Three of them can be pointed out :

a) specific inhibition of T or B cells or appearance of T suppressor cells, is suggested by recent experiments in the rat infected with *S. mansoni* ;

b) antigenic competition ;

c) non specific inhibition. There is some preliminary evidence that schistosomes might release non specific factors apt to block effector mechanisms.

2) *Host antigens*

It is now clearly established that antigens originating from the host do exist in schistosomes. Two kinds of host antigens must be distinguished : glyco-

proteins or alpha 2 macroglobulin actively synthetized by the parasite, and acquired antigens located on the schistosome surface with blood group specificities.

A clear-cut demonstration has not yet been provided that these antigens, wherever they come from, have a prominent role in protecting the parasite from the immune factor mechanism. It is likely, however, that endogenous host antigens decrease the immunogenicity of the schistosome, while acquired antigens on the parasitic surface block the antigenic specificities of the adult worm and accordingly protect it from the immune response.

3) Soluble antigens

The soluble antigens previously mentioned can contribute to mechanisms of survival in various ways : antigenic competition, induction of tolerance, stimulation of T suppressor cells, neutralization of high affinity antibodies and formation of circulating immune complexes with immunosuppressive effect. All of these hypothetical mechanisms, which now have been extensively investigated, seem to contribute to a feed-back regulation allowing the parasite to survive in the immunized host.

In the light of this short review it appears that the way to successful vaccination will be long and difficult. Schistosomes represent formidable adversaries for immunologists. The adaptative abilities of these flukes might defeat for some time yet the practical applications of recently gained knowledge of effector immune mechanisms. It is evident, however, that if one compares the 10-year-old groundings in the field and the recent progress in the characterization of antigens, effector mechanisms, mechanisms of survival or immunopathology, one may feel optimistic about the ultimate outcome of these researches and the future possibility of the immunological control of schistosomiasis.

THE ROLE OF HYPERSENSITIVITY IN THE DEVELOPMENT
OF PATHOLOGICAL LESIONS IN BILHARZIAL INFECTION*

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Dr. Abdallah, distinguished colleagues, ladies and gentlemen : In carrying out the mandate of His Excellency, Dr. Fouad Mohieddin, the Minister of Health of the Arab Republic of Egypt, we must attempt to determine the contributions that each of our respective scientific disciplines has to offer for alleviating and preventing human schistosomiasis.

In this respect, we can only state that immunology has great potential value, but up to the present time, despite a substantial investment of talent and energy, it has rendered comparatively little benefit to those millions of people suffering from schistosomiasis that all members of this conference, regardless of their national or professional background, would want to restore to, and maintain in, better health. Studies of human and experimental pathology and of immunology have helped us to understand better the mechanisms of host defense and of pathogenesis of schistosomiasis but, from a practical point of view, these studies must still be regarded as a speculative investment made with our eyes towards the possibilities of the future rather than toward the realities of the present. Yet, we are not talking about the distant future :

Hoffman (1975) has estimated that it should be possible, within the next 5 to 10 years, to know whether an effective human vaccine against schistosomiasis is feasible and whether it should be a live or a non-living vaccine. Drs. R. Smithers and M. Taylor will also have further comments on this matter. There is the possibility too that immunological tests may be useful for categorizing the disease states of schistosome-infected patients, and thus aid us in their medical management. On the other hand, in the words of my wise friend and colleague, Zilton Andrade, we cannot be sure as yet that immunology has anything positive to contribute to schistosomiasis, but we can be sure that schistosomiasis has much to contribute to immunology, as a scientific discipline.

In considering the role of hypersensitivity in schistosomiasis, we must distinguish three categories of phenomena :

1) Immediate or reaginic hypersensitivity which is mediated by homocytotropic antibodies, acting on mast cells

2) Antigen-antibody complexes which lead to the activation of the complement cascade, giving rise to local or generalized Arthus type reactions, and

3) Delayed hypersensitivity, often designated as cell-mediated immunity (CMI), which is elicited by sensitized lymphocytes and their products, named «Lymphokines».

These three categories of responses are neatly separable from each other only in the immunological laboratory, but in the course of an infectious disease process such as schistosomiasis they appear in successive and mixed form and are often difficult to distinguish from each other.

While it is true that the disease manifestations of schistosomiasis are closely related to immunological host responses, there are also indications that these responses are, at least in part, beneficial to the host. We cannot, therefore, assume that abolishing host immune reactivity by drugs or by other ablative maneuvers will necessarily improve the health of a patient. Two recent reports support this statement : The clinical finding by Queiroz et al. (1973) that cyclophosphamide had no beneficial effect on schistosomal nephropathy associated with *Schistosoma mansoni* in Brazil, and the experimental finding by Buchanan et al. (1973) that total ablation of T-lymphocytes in mice by thymectomy and lethal radiation increased their mortality after *S. mansoni* infection. Doubtlessly, our Chairman, Professor A. Capron, can comment more competently on this matter, but to me these reports suggest that, *before one tampers with the immunological reactivity of a bilharzial patient, one must be well aware of the rationale of such intervention, and of all of its possible side effects,* the more so, since schistosomicidal drugs are available to us the efficacy and side effects of which are already well known (von Lichtenberg, 1975).

We shall now briefly review the role of hypersensitivity at three successive stages of schistosome infection : 1) the prepatent stage, 2) the acute stage and 3) the chronic stage.

1) *In the Prepatent Stage*

Penetration of schistosome cercariae produces a dermatitis which is boosted after repeat exposure. In mice, some mast cell degranulation occurs in both primary and secondary skin responses, and the timing and morphology of the peak response in sensitized mice at 6-24 hr suggest an antibody-mediated process (Murell et al., 1975). Sher et al. (1975) have further established that immunity to schistosomula is depressed in mice deficient in complement fractions C'3 and C'5.

By using the Dominici staining method specific for eosinophil granules, our group has recently shown that a highly significant eosinophil response to schistosomula occurs in the skin of immunized mice even after single re-challenge (von Lichtenberg et al., 1976 b). Note that the increase in number of dermal eosinophils is of the order of 7-10 fold in the immune mice. This is further illustrated in Fig. 1 which shows representative eosinophilia in an immunized mouse challenged with cercariae as seen 20 hr after exposure. Currently we are engaged in experimental studies to ascertain how this eosinophil response is mediated, since it appears to be closely linked both to hypersensitivity and to immunity itself : Dr. Adel Mahmoud will further discuss this matter in a later session, in the light of recent findings by his group that anti-eosinophil serum significantly suppresses immunity in mice *in vivo* (Mahmoud et al., 1975). Dr. Mahmoud, like other Egyptian investigators before him, is today working at the very frontiers of immunological science.
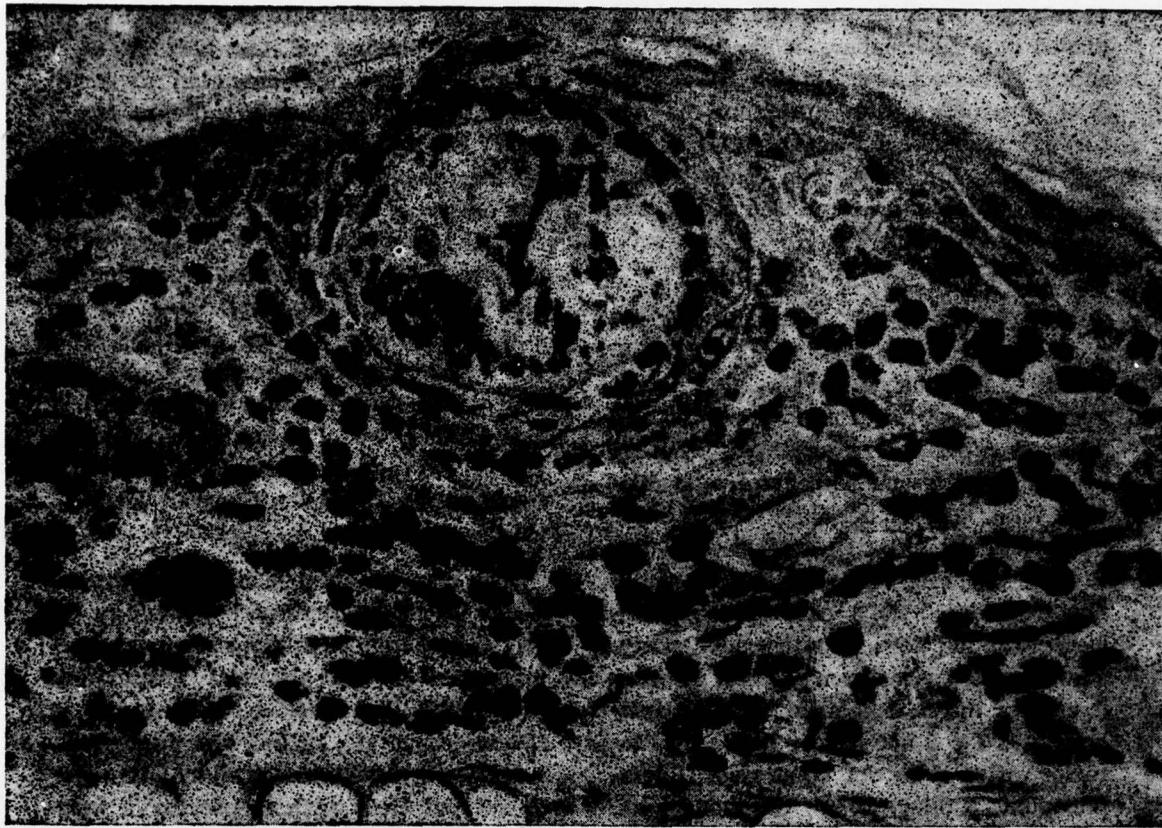
Fig. 1. Intraepidermal schistosomulum in an immune mouse, 24 hours after exposure to *S. mansoni* cercariae. A large proportion of the cells with segmented nuclei streaming toward and surrounding the schistosomulum are eosinophils, as shown with the Dominici stain. The cartilage of the ear pinna is seen at the border of the figure. (X600).

2) Acute Stage

During acute schistosomiasis, coinciding with early oviposition 1-4 months after exposure, allergic manifestations may be prominent, especially in severe human infection («toxemic stage», «Katayama fever»). Tissue reactions to eggs are florid during that period, and in experimental chimpanzees the earliest lesions of pipe stem fibrosis and of nephropathy are detected. CMI responses attain a maximal level, followed by a rise of hemagglutinating and immunofluorescent antibody. This is regarded as the stage of maximal host immunological reactivity.

When acute schistosomiasis occurs, more frequently in non-residents than in residents of an endemic area, there is eosinophilia, mild splenomegaly, and fever, a syndrome which has been compared with serum sickness, and which contrasts sharply with the ravages of late pipe stem fibrosis and portal hypertension. Acute-stage liver granulomas of *S. mansoni* are characterized by large size, intense eosinophilia, central necrosis and the Hoeppli phenomenon which we believe to be an antigen-antibody complex analogous to the circum-oval precipitate which can be produced *in vitro* as a result of interaction between host serum and the

secretory products of the miracidial glands (von Lichtenberg et al., 1966). As shown by histochemical stains, these antigenic products can escape from the egg through the submicroscopic pores of the egg shell (Smith & von Lichtenberg, 1967), and become sequestered in the granuloma center.

3). *Chronic Stage*

In the transition from acute to chronic schistosomiasis the host's CMI responses to egg antigens are weakened, and granuloma size is reduced ; levels of egg directed antibody are increased, whereas heat-labile, classical homocytotropic antibody fades. The immunology of this event named «Endogenous Desensitization» by Warren (Domingo & Warren, 1968) and now considered as a case of «Immunological Modulation» is still under study, and no decision has been reached whether it resides in an imbalance between antibody and T-cell activity or in the proliferation of a clone of suppressor T-lymphocytes (Colley, 1976 ; Gershon et al., 1974).

Age-related autopsy observations in human schistosomiasis haematobia have shown that the most florid and exudative urinary mucosal lesions tend to occur in the young, while the bladders of older patients often show only minimal tissue response («burnt-out lesions»). This progression was clearly demonstrated in a collaborative study of a Cairo series of about 200 autopsies by Dr. I. Kamel, Anwar Elwi, here present, and by Dr. J.H. Smith (Smith et al., 1975). A precipitous fall in histological activity was noted around 40 years of age. Statistical observations of this kind must be related to schistosome egg production patterns, as well as to host immunological reactivity, and it is not yet clear whether they are analogous to the experimental findings in animals. Thus, in the chimpanzee, we

were able to illustrate the formation of polypoid patches of the bladder at 7 months of infection, changing to sandy patches by the 18th month (Sadun et al., 1970) but in man the decline of exudative bladder changes appears to take a number of years, and may possibly be more related to asynchronous egg laying or to the death of adult worms. Worm loss is also the most likely explanation of the statistical decline of egg excretion with age despite persistent prevalence of infection which was demonstrated so well in the studies by Bradley & McCullough (1973). These matters will be further taken up by Dr. Allen Cheever, later in this session.

In experimental schistosome granulomas («Pseudotubercles») elicited by infections of eggs of *S. mansoni* and *S. haematobium* or in artificial granulomas produced with particle-associated antigen (Dunsford et al., 1974) the anamnestic response is clearly mediated by CMI ; this is not yet clear with respect to *S. japonicum* (Warren et al., 1975 ; von Lichtenberg et al., 1973). Moreover, in chronic infection, β-lymphocytes and antigen-antibody complexes have been demonstrated in schistosome granulomas by some workers. We postulated some time ago that the host immune response results in accelerated egg antigen sequestration and destruction in granulomas (von Lichtenberg, 1964).

When viable eggs are injected into the pulmonary vessels, immunofluorescent stainable antigen first diffuses rather freely, but, within a few days, antigen is concentrated around the egg shell and in the granuloma center. We also know that some of the lipid fractions isolated from eggs are lysophosphatides which are known to be generally toxic to cytoplasmic membranes (Simth et al., 1971) and this brings up the question of

whether an adjuvant material is produced by schistosome eggs. Recently, we have shown that granulomas averaging 8-10 times the normal size can be elicited by pre-injecting mice with lentinan, a polysaccharide derived from *Lentinus edodes,* an edible Japanese mushroom which is known to act as a T-cell adjuvant (von Lichtenberg et al., 1976 a). The question of the protective significance of granulomatous hypersensitivity therefore deserves further investigation. With respect to the chronic stage of schistosomiasis, studies in chimpanzees have suggested that granuloma formation is not directly responsible for pipe stem fibrosis, or for schistosomal nephropathy. The pathogenesis of these latter lesions remains unknown, but host sensitization is likely to have an important role.

Chimpanzees heavily infected with *S. japonicum* or *S. mansoni* develop striking pipe stem fibrosis simlar to the human with abdominal collateral circulation and a marked rise in serum immunoglobulin. In this model, pipe stem fibrosis begins as a diffuse inflammatory change in middle sized and large portal fields, and within a few months after exposure dense collagen is laid down in these triads in a diffuse pattern. This is accompanied by widespread obliterative endophlebitis and by compensatory arterial dilatation and hyperplasia. No eggs or granulomas are found where these early lesions take place, but the pipe stem lesions are located proximal to smaller portal triads in which eggs and granulomas are numerous at this time (von Lichtenberg et al., 1971). Pipe stem fibrosis results in the obliteration and multiple anomalies of larger portal veins which are unique to man and to chimpanzees and are not seen in small animal experimental models. In the chimpanzee model, the occurrence of glomerular lesions depends on the inten-

sity of infection and is somewhat parallel to that of pipe stem fibrosis, as was also found in human *S. mansoni* infection in Brazil (Cavallo et al., 1974). The glomerular lesion in chimpanzees is a striking proliferation of mesangial cells and matrix associated with hyaline droplets. Some animals with nephropathy also showed a focal arteritis characterized by fibrinoid necrosis and hyaline droplets, and their abdominal lymph nodes were altered by follicular hyperplastic and plasma cell proliferation. Several authors have described mesangial or basement membrane deposits in schistosomal nephropathy, as well as IgG and complement deposition ; it is not clear, however, whether immune complexes are formed with egg antigens (SEA), or with adult circulating antigens which Nash (1974) and our group (von Lichtenberg et al., 1974) have shown by specific immunofluorescence to originate from the gut of schistosomes, or even with endogenous host tissue products. The relatively benign clinical course of schistosomal nephropathy compared with classical immune complex nephritis also remains unexplained. Drs. Madwar and Higashi are both interested in the relationship of nephropathy to antigen-antibody complexes and will report on their studies later in this conference. Sadun et al. (1974) have recently shown that nephropathy is substantially improved when chimpanzees are treated for *S. japonicum* infection with Sq-18506 which arrests their liver fibrosis. In these animals, only minimal glomerular lesions persisted. On the other hand, portocaval shunting while also arresting liver fibrosis, still permits nephropathy to continue actively (Sadun et al., 1975). (Unfortunately, the best available animal model for both schistosomal pipe stem fibrosis and nephropathy, the chimpanzee, has now become unavailable to most investigators, but other useful ex-

perimental models are still available for study).

There is good evidence that schistosome granuloma size can be reduced by a variety of immunosuppressive drugs, including choleratoxin (Warren et al., 1974) and, particularly, by niridazole (Mahmoud & Warren, 1974) but, except in the latter case, we do not know whether granuloma suppression will prevent either pipe stem liver fibrosis, or indeed any of the lesions which account for most of the clinical morbidity in schistosomiasis mansoni and japonica. Therapeutic experiments with immunosuppressive agents should therefore be undertaken only with caution, if at all.

A better rationale exists for induction of *specific* rather than of general immunological unresponsiveness, particularly during the early «toxemic» stage of schistosomiasis. This might be feasible if the principal egg antigens could be sufficiently purified for clinical use ; the antigen (SEA) available at present is rather crude. Progress in this direction is being made by (Hamburger et al., 1976) and by Brown & David (unpublished).

I realize that I have submitted you to a rather concentrated and superficial survey of a very complex field of research and want to thank our chairman and this audience for bearing with me during this difficult task.

LITERATURE CITED

Bradley, D.J. & McCullough, F.S., 1973. Egg output stability and the epidemiology of Schistosoma haematobium II. An analysis of the epidemiology of endemic S. haematobium. Trans. r. Soc. trop. Med. Hyg. 67: 491-500.

Buchanan, R.D., Fine, D.P. & Colley, D.G., 1973. Schistosoma mansoni infection in mice depleted of thymus-dependent lymphocytes. Am. J. Path. 71: 207-218.

Cavallo, T., Galvanek, E.G., Ward, P.A. & von Lichtenberg, F., 1974. The nephropathy of experimental hepatosplenic schistosomiasis. Amer. J. Path. 76: 433-450.

Colley, D.G., 1976. Adoptive suppression of granuloma formation. J. exptl. Med. 143: 696-700.

Domingo, E.O. & Warren, K.S., 1968. Endogenous desensitization: Changing host granulomatous response to schistosome eggs at different stages of infection with Schistosoma mansoni. Amer. J. Path. 52: 369-379.

Dunsford, H.A., Lucia, H.L., Doughty, B.L. & von Lichtenberg, F., 1974. Artificial granulomas from bentonite and latex carrier particles. Amer. J. trop. Med. Hyg. 23: 203-217.

Gershon, R.K., Lance, E.M. & Kondo, K., 1974. Immuno-regulatory role of spleen localizing thymocytes. J. Immunol. 112: 546-554.

Hamburger, G., Pelley, R.P. & Warren, K.S., 1976. Purified Schistosoma mansoni egg antigens: Determination of the stage and species specificity of their serological reactivity by radioimmunoassay. Submitted, J. Immunol.

Hoffman, D.B., Jr., 1975. The strategic plan for schistosomiasis research. Edna McConnell Clark Foundation, New York, N.Y. June 1975, 41 pp.

Mahmoud, A.A.F. & Warren, K.S., 1974. Anti-inflammatory effects of tartar emetic and niridazole: Suppression of schistosome egg granuloma. J. Immunol. 112: 222-228.

Mahmoud, A.A.F., Warren, K.S. & Peters, P.A., 1975. A role for the eosinophil in acquired resistance to Schistosoma mansoni infection as determined by anti-eosinophil serum. J. expt. Med. 142: 805-813.

Murrell, K.D., Dean, D.A. & Stafford, E.E., 1975. Resistance to infection with Schistosoma mansoni after immunization with worm extracts or live cercariae: Role of cytotoxic antibody in mice and guinea pigs. Amer. J. trop. Med. Hyg. 24: 955-962.

Nash, T.E., 1974. Localization of the circulating antigen within the gut of Schistosoma mansoni. Amer. J. trop. Med. Hyg. 23: 1085-1087.

Queiroz, F.P., Brito, E., Martinelli, R. & Rocha, H., 1973. Nephrotic syndrome in patients with Schistosoma mansoni infection. Amer. J. trop. Med. Hyg. 22: 622-628.

Sadun, E.H., von Lichtenberg, F., Erickson, D.G., Cheever, A.W., Bueding, E. & Anderson, J.S., 1974. Effects of chemotherapy on the evolution of schistosomiasis japonica in chimpanzees. Amer. J. trop. Med. Hyg. 23: 639-661.

Sadun, E.H., Reid, W.A., Cheever, A.W., Duvall, R., Swan, K.G., Kent, K.M., Bruce, J.I. & von Lichtenberg, F., 1975. Effects of portocaval shunting on Schistosoma japonicum infection in chimpanzees: Dissociation of pipe stem fibrosis and glomerulopathy. Amer. J. trop. Med. Hgy. 24: 619-631.

Sadun, E.H., von Lichtenberg, F., Cheever, A.W., Erickson, D.G. & Hickman, R.L., 1970. Experimental infection with Schistosoma haematobium in chimpanzees: Parasitologic, clinical, serologic and pathological observations. Amer. J. trop. Med. Hyg. 19: 427-458.

Sher, A., Knopf, P.M., Gibbons, N., Doughty, B.L. & von Lichtenberg, F., 1975. Mechanism of acquired immunity to S. mansoni in the laboratory mouse. Proc. 10th Conf. on Parasitic Disease, U.S.-Japan Cooperative Medical Science Program, U.S.D.H.E.W., Bethesda, Md., pp. 132-133.

Smith, J.H. & von Lichtenberg, F., 1967. The Hoeppli phenomenon in schistosomiasis. Amer. J. Path. 50: 993-1007.

Smith, J.H., Kamel, I.A., Elwi, A. & von Lichtenberg, F., 1975. A quantitative post-mortem analysis of urinary schistosomiasis in Egypt. I. Pathology and Pathogenesis. Amer. J. trop. Med. Hyg. 23: 1054-1071, 1974; II. Evolution and Epidemiology. ibid. 24: 806-822.

Smith, T.M., Lucia, H.L., Doughty, B.L. & von Lichtenberg, F., 1971. The role of phospholipids in schistosome granulomas. J. infect. Dis. 125: 629-639.

von Lichtenberg, F., 1964. Studies on granuloma formation III. Antigen sequestration and destruction in the schistosome pseudotubercle. Amer. J. Path. 45: 75-94.

von Lichtenberg, F., 1975. Schistosomiasis as a world problem : Pathology. J. Toxicol. Envtl. Hlth. 1: 175-184.

von Lichtenberg, F., Bawden, M.P. & Shealey, S.H., 1974. Origin of circulating antigen from the schistosome gut. An immunofluorescent study. Amer. J. trop. Med. Hyg. 23: 1088-1091.

von Lichtenberg, F., Erickson, D.G. & Sadun, E.H., 1973. Comparative histopathology of schistosome granulomas in the hamster. Amer. J. Path. 72: 149-178.

von Lichtenberg, F., Sadun, E.H., Cheever, A.W., Erickson, D.G., Johnson, A.J. & Boyce, H.W., 1971. Experimental infection with Schistosoma japonicum in chimpanzees. Parasitologic, clinical, serologic and pathological observations. Amer. J. trop. Med. Hyg. 20: 850-893.

von Lichtenberg, F., Sher, F.A., Byram, J.E. & Joel, M., 1976a. Potentiation of granulomatous inflammation by Lentinan. Amer. J. Path. 82: 67a (ABS.).

von Lichtenberg, F., Smith, J.H. & Cheever, A.W., 1966. The Hoeppli phenomenon in schistosomiasis. Comparative pathology and immunopathology. Amer. J. trop. Med. Hyg. 15: 886-895.

von Lichtenberg, F., Sher, A., Gibbons, N. & Doughty, B.L., 1976b. Eosinophil-enriched inflammatory response to schistosomula in the skin of mice immune to Schistosoma mansoni. Amer. J. Path. 84: in Press Aug.

Warren, K.S., Boros, D.L., Hang, L.M. & Mahmoud, A.A.F., 1975. The Schistosoma japonicum egg granuloma. Amer. J. Path. 80: 279-294.

Warren, K.S., Mahmoud, A.A.F., Boros, D.L., Rall, T.W., Mandel, M.A. & Carpenter, C.C.J., Jr., 1974. In vivo suppression by cholera toxin of cell mediated and foreign body inflammatory responses. J. Immunol. 112: 996-1007.

INTENSITY OF INFECTION AS RELATED TO PATHOLOGICAL LESIONS IN BILHARZIAL PATIENTS

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The work of J. Allen Scott (1938) in Egypt first indicated that the passage of *S. mansoni* eggs in the stool was relatively constant and might serve as a quantitative index of infection. The pioneering studies of Kloetzel (1963) in Brazil indicated a relationship between the intensity of infection and disease.

Our present study is based on 398 consecutive autopsies performed in the Department of Pathology of Kasr el Ainy Hospital in Cairo (Table 1). No cases were found infected with only *Schistosoma mansoni*; 98 were infected with both *S. mansoni* and *S. haematobium*, 160 with only *S. haematobium* and 140 were uninfected.

The only clinically significant liver disease associated with schistosomiasis was Symmers' clay pipestem fibrosis of the liver (Table 2). This diagnosis was made on gross examination and confirmed microscopically. It is also possible to make this diagnosis from a surgical wedge biopsy of the liver, but we would emphasize that it is seldom, if ever, possible to diagnose Symmers' fibrosis from a needle biopsy of the liver.

Symmers' fibrosis was seen only in cases infected with *S. mansoni*, and the intensity of *S. mansoni* infection was much greater in cases with Symmers' fibrosis than in the remaining cases (Table 3). Symmers' fibrosis was the cause of death in 8 cases. The number of worm pairs recovered from cases of Symmers' fibrosis was very similar to the number recovered at autopsy from Brazilian cases of Symmers' fibrosis (Cheever, 1968), suggesting that neither the difference in worm strains nor in the human host had a marked effect on the development of Symmers' fibrosis in these two countries.

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TABLE 1. Background data for cases studied at autopsy.

	Uninfected	All infected cases	Infected with S. mansoni and S. haematobium*	Infected with S. haematobium only*
Number of cases	140	258	98	160
Mean age	38	41	37	43
Geometric mean of S. haematobium eggs recovered**	0	100,000	1,000,000	25,000

* These are subgroups of the group designated "all infected cases". No pure S. mansoni infections were found.

** The geometric mean is for all eggs recovered, principally from the bladder, ureters, seminal vesicles, intestines, lungs and liver.

TABLE 2. Prevalence of various types of severe liver disease in infected and uninfected cases

Condition of liver	Uninfected	All infected cases	Infected with S. mansoni and S. haematobium	Infected with S. haematobium only
Symmers' fibrosis	0%	4.3%*	11.2%**	0%
Cirrhosis	7.1	9.3	6.1	11.3
Other liver disease	1.4	1.9	1.0	2.5

* Significantly different from uninfected group (p <.05) in 2 chi square test.
** Significantly different from uninfected group (p <.01) in 2 by 2 chi square test.

TABLE 3. Intensity of Schistosoma mansoni infection in Symmers' fibrosis.

	Percentage of cases with more than 160 female S. mansoni recovered	
	Egypt	Brazil
Symmers' fibrosis	44%	37%
Other S. mansoni infected cases	6%	4%

Cirrhosis was not significantly more frequent in infected patients. Other severe liver disease (subacute yellow atrophy, jaundice or fibrosis of unknown etiology) was also not significantly more frequent in infected than in uninfected cases. Microscopic hepatic fibrosis in cases without severe liver disease was more frequent in infected cases and was clearly related to the number of eggs in the liver ; however, this microscopic fibrosis was not associated with splenomegaly or other signs of portal hypertension, and we doubt that it was of clinical significance. We thus found no group which might correspond to that of «fine bilharzial periportal fibrosis», for these cases described by Hashem (1947) had splenomegaly and portal hypertension equivalent to that seen in Symmers' fibrosis.

In summary, all cases of bilharzial hepatosplenic disease in our series had classical Symmers' clay pipestem fibrosis of the liver, and all cases with Symmers' fibrosis were infected with *S. mansoni*. The intensity of *S. mansoni* infection in these cases was similar to that seen in Brazil and was much greater than the intensity of infection in other cases.

Most *S. haematobium* infections were inactive, and adult female *S. haematobium* worms were recovered in only 17% of infected cases. Large numbers of *S. haematobium* eggs were often present in the tissues of inactive cases, suggesting a slow rate of clearance of eggs from the tissues. *S. mansoni* females were recovered in 62% of *S. mansoni* infected cases, and large numbers of *S. mansoni* eggs were rarely seen in the absence of active infection.

Severe colonic polyposis occurred only in *S. mansoni* infected cases, was the apparent cause of death in two cases and was clearly related to the intensity of *S. mansoni* infection. Occasional colonic polyps were also seen in individuals infected only with *S. haematobium*. No clue was found to explain the frequency of colonic polyposis in Egypt and its virtual absence in Brazil. The intensity of *S. mansoni* infection was comparable and the relative distribution of *S. mansoni* eggs in the liver, lungs and intestines did not differ significantly in Brazilian and Egyptian cases.

Our material, like that studied previously in this hospital by Smith et al. (1974), showed no relation between the presence of glomerulonephritis and *S. haematobium* infection. Less marked glomerular changes, such as mesangial cell proliferation and mesangial thickening, were similarly unassociated with infection (Sadigursky et al., submitted for publication). This is in marked contrast to the apparent frequency of glomerulonephritis noted in Upper Egypt by Ezzat et al. (1974).

Death in six individuals was attributed, on morphologic grounds, to obstructive schistosomal uropathy. Several deaths from obstructive uropathy, usually caused by benign prostatic hypertrophy, occurred in the control group, and no overall difference in fatal obstructive uropathy was found between infected and uninfected cases. Six individuals, all infected, died of bladder cancer. In aggregate, Symmers' fibrosis, schistosomal colonic polyposis, schistosomal obstructive uropathy and bladder cancer associated with schistosome infection were the cause of death in 8.5% of infected cases and 5.5% of all autopsies. It is possible that schistosome infections contributed to death in less clearcut ways in other cases, but it is evident that substantial mortality was present in this series even when considering only these major complications of the disease.

We believe our series of cases to be free of selection of cases after death. We cannot assess the selective factors which may determine admission to the Hospital. Our findings, from an urban hospital, may underestimate or overestimate the impact of disease caused by schistosomiasis. This emphasizes the need for studies in the field, from which a more meaningful assessment of impact might be made. The strengths of autopsy studies include the ability to evaluate the morphologic chan-

ges accurately, to see clinically unsuspected disease and to determine the cause of death in most cases.

LITERATURE CITED

Cheever, A.W., 1968. A quantitative post mortem study of schistosomiasis mansoni in man. Amer. J. trop. Med. Hyg., 17: 38-64.

Ezzat, E., Osman, R.A., Ahmet, K.Y. & Soothill, J.F., 1974. The association between Schistosoma haematobium infection and heavy proteinuria. Trans. roy. Soc. trop. Med. Hyg., 68: 315-318.

Hashem, M., 1947. The etiology and pathogenesis of the endemic form of hepato-splenomegaly «Egyptian splenomegaly». J. Egypt. med. Assoc., 30: 48-79.

Kloetzel, K., 1963. Some quantitative aspects of diagnosis and epidemiology in schistosomiasis mansoni. Amer. J. trop. Med. Hyg., 12: 334-337.

Sadigursky, M., Kamel, I.A., Elwi, A.M., Danner, R., Cheever, A.W. & Andrade, Z.A., (in press).

Absence of schistosomal glomerulopathy in Schistosoma haematobium infection in man. Trans. roy. Soc. trop. Med. Hyg.

Scott, J.A., 1938. The regularity of egg output of helminth infestations with special reference to Schistosoma mansoni. Amer. J. Hyg., 27: 155-175.

Smith, J.H., Kamel, I.A., Elwi, A. & von Lichtenberg, F., 1974. A quantitative post mortem analysis of urinary schistosomiasis in Egypt. I. Pathology and pathogenesis. Amer. J. trop. Med. Hyg., 23: 1054-1071.

Smith, J.H., Elwi, A.M., Kamel, I.A. & von Lichtenberg, F., 1975. A quantitative post mortem analysis of urinary schistosomiasis in Egypt. II. Evolution and epidemiology. Amer. J. trop. Med. Hyg., 24: 806-822.

CONCOMITANT IMMUNITY IN SCHISTOSOMIASIS

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Everyone will accept that immunity develops after an attack of measles. Not everyone will agree that immunity develops after exposure to schistosomes. Measles is an acute disease ; if the patient recovers he is completely resistant to further infection. Schistosomiasis is a chronic disease ; it may afflict a patient for most of his life ; how can there be any acquired immunity in such circumstances ?

In this paper I hope to show that in experimental hosts and probably also in man, immunity to reinfection does occur in schistosomiasis although at the same time the host may be unable to destroy his population of established adult worms. I wish to emphasize that schistosomiasis is not an example of sterile immunity where, if the host survives, there is complete destruction of the invading organisms followed by resistance to reinfection. Immunity in schistosomiasis is better regarded as a delicately balanced relationship between the immunological forces of the host on the one hand, and the circumvention of these forces by the parasite, on the other. This balance enables the extended survival of both host and parasite.

Our knowledge of immunity to schistosomes has come exclusively from experimental animal models ; there is still very little known about immunity in man. In recent years the mouse has served as a useful experimental animal and I should like to illustrate from studies in the mouse, some of the characteristics of schistosome immunity. As in the human, *Schistosoma mansoni* will cause a chronic infection in the mouse. After exposure to 20-30 cercariae, egg-laying worms persist throughout the life of the mouse and there is development of hepatosplenic disease.

Resistance to schistosomiasis cannot be measured by antibodies ; it can only be measured by following the fate of a challenge infection. In mice this is done by perfusing the worms from the liver 6 weeks after the challenge. Enumeration of the worms of the challenge which survive has shown that mice develop immunity several weeks after a primary infection. But a more rapid assay of immunity is to recover the young schistosomes or schistosomula, as they are called, as they pass through the lungs 4-5 days after exposing the skin to cercariae. This is easily done by removing the lungs, mincing them finely with scissors and incubating them in saline. The young schistosomula migrate out of the pieces of chopped lung and can be counted under the microscope (Sher et al., 1974a).

Peak numbers of schistosomula are recovered from the lungs of normal mice 4-5 days after exposure to cercariae. When schistosomula are recovered from the lungs of previously infected mice, *i.e.*

mice which have developed immunity, there is a clear reduction in the numbers recovered. This is evidence that at least some of the challenge infection has been destroyed in the lungs or at an earlier stage in the pathway of migration. When this decrease in recovery is measured, say on day 5, it provides a quantitative measure of the degree of immunity which has developed.

This assay of immunity is rapid; the results are available only 5 days after challenge, and enable us to follow the development of acquired immunity in the mouse after a primary infection. Figure 1 illustrates the results of an experiment in which a number of mice were all exposed to 30 cercariae; at regular intervals following this primary infection, groups of the mice were challenged and the lung recovery method was used to determine the resistance which had developed. It is clear that resistance begins to appear about 7 weeks after infection and reaches a plateau about 20 weeks later, when about 80% of a challenge infection is destroyed.

It is obvious that this immunity differs from the classical concept of sterile immunity; even at the period when 80% of a challenge infection is destroyed by the mouse, the worms from the initial exposure are still present and laying eggs.

Thus we have immunity in the presence of an active infection and to describe this situation we have used the term concomitant immunity (Smithers & Terry, 1969). This term is borrowed from tumour immunology where animals bearing one tumour were sometimes resistant to a second graft. The new tumour was not accepted even though the first tumour continued to grow progressively. By analogy, invading schistosomula are destroyed by the immune response of the host while the adult worms remain unaffected.

This situation is also clearly seen in the rhesus monkey. Adult schistosomes have been transferred surgically into the mesenteric veins of normal monkeys. The transferred worms produce eggs which
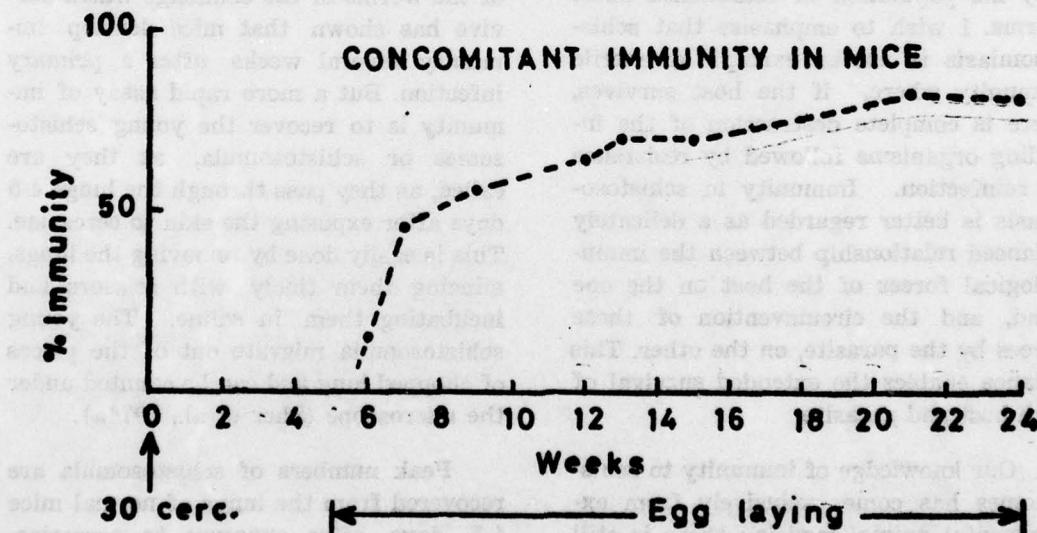
Fig. 1. Mice were exposed to 30 cercariae; at various intervals following exposure, groups of 5 mice were challenged with 500 cercariae and immunity was assayed by the lung recovery technique. (After Sher et al., 1974a).

are passed in the faeces. When these monkeys are challenged with cercariae several weeks later, it is clear that they have developed resistance to the challenge (Smithers & Terry, 1967). It is the presence of the transferred worms which has induced this immunity ; yet, paradoxically, these worms are still present and passing eggs, unaffected by the immunity they have engendered. Once again we have an example of concomitant immunity.

If we are to understand concomitant immunity, it will be necessary to answer two questions ; first, what is the mechanism which kills the schistosomula of a challenge and what are the antigens involved ? Secondly, how is the adult worm able to circumvent the immunity of the host and remain unharmed in the bloodstream ?

These problems are beginning to be solved. First, it now appears that antibodies play a role in resistance. This has been shown by the ability to passively transfer protection with the serum from immune mice, or immune rats (Sher et al., 1975 ; Phillips et al., 1975) although in mice only about half the immunity of the donor can be transferred with serum alone.

There is also evidence that cells as well as antibodies are involved in protective immunity. There are several in vitro systems which kill or damage young schistosomes in culture either by antibody plus complement or by a combination of antibody and cells (Clegg & Smithers, 1972 ; Dean et al., 1974 ; Capron et al., 1975). A word of warning ; these in vitro immune systems do not necessarily correlate with immunity in vivo. For example, one can vaccinate a host with schistosome antigen so that the animal produces an antibody that is lethal

to young schistosomes in culture. Despite this in vitro phenomenon, however, the animal remains susceptible to infection (Sher et al., 1974b).

Nevertheless, one particular in vitro system described by Butterworth and his colleagues (1974) in Nairobi, demands special attention. In this system, serum from human schistosome patients damages schistosomula in culture when in combination with peripheral white blood cells from normal humans. The serum factor is an antibody of the IgG class and the cells involved are identified as eosinophils (Butterworth et al., 1975).

This important finding has led Mahmoud and his colleague in Cleveland to investigate the role of the eosinophil in vivo. They used a battery of specific antisera directed against the various white blood cells of mice to identify the cell type involved in mouse immunity (Mahmoud et al., 1975). Their results clearly demonstrate the role of the eosinophil in the effector mechanism ; lymphocytes, neutrophils and macrophages do not appear to be involved.

The function of the eosinophil has been the subject of debate for many years. Have the parasitologists finally discovered a role for this cell in worm immunity ?

The second problem, to identify the mechanism which enables the worm to circumvent the host's immunity, is still to be solved. There are several possible theories and almost certainly more than one mechanism is operating.

Examination of the worm's surface should provide an important insight into this problem. Electron micrographs of the surface of S. mansoni show that the tegument is bounded by a plasma membrane, but it is an unusual membrane in that

it is multilaminate. The cercaria has a conventional trilaminate surface membrane; the multilaminate membrane of the adult is developed in the dermis within 2 hr of penetration (Hockley & McLaren, 1973). Electron micrographs of the adult surface often suggest that small segments of the membrane are being sloughed off into the bloodstream. There is additional evidence from isotope studies that the surface membrane turns over and particulate membrane antigens are released by the worm into the environment (Kusel et al., 1975). Such evidence suggests that the surface membrane is being continuously replaced. Continuous replacement could be a device to counteract damage by an immune reaction.

In vitro experiments have added support to this idea; when adult schistosomes are exposed to an antibody directed against surface components there is an intense activity of the surface membrane. It appears that fragments of membrane are being cast off and that the damaged membrane is being removed (Perez & Terry, 1973). This phenomenon may represent an important defence mechanism against the host's immune attack.

Other factors which may be involved in the escape mechanism are host antigens. These are antigens which are shared in common by host and parasite; they have been conclusively demonstrated in schistosomes (Smithers & Terry, 1976). Some host antigens are of parasite origin and have evolved under selective pressure to resemble antigens of the host. These endogenously synthesized host antigens will undoubtedly reduce the overall immunogenicity of the parasite and favour its survival. Other host antigens are actually acquired from the host and incorporated into the schistosome's surface. These acquired host molecules are believed to be glycolipids related to the A, B and H blood group substances. For example, schistosomula maintained in human blood of type A can be shown to acquire the A blood group antigen on its surface (Clegg, 1974). The possible role of these surface acquired host antigens in effecting an immunological disguise of the adult worm is axiomatic, although as yet there is no direct evidence of their protective role.

So much for recent experimental work, but what of the situation in man? The concept of concomitant immunity has now been established in a number of experimental animal models and as far as one can extrapolate from experimental hosts to man, one would expect concomitant immunity to be developed by man against his schistosomes. In other words, a patient who is passing eggs may still be able to resist further infection with cercariae.

It is not possible to do well-controlled infection and challenge experiments in man and most of our knowledge must of necessity come from epidemiological studies. Evidence of concomitant immunity in man is still arguable. In most communities studied in detail, the peak of intensity and prevalence usually occurs in the second decade of life and is followed by a gradual decline. It may be that this decline represents the acquisition of concomitant immunity, but such data can also be explained in terms of decreasing contact with infected water in the older age groups.

There are a few examples however which give a clearer indication of the development of concomitant immunity in man. I am thinking particularly of the work of Kloetzel & Da Silva (1967) in Brazil. They studied a group of immigrants who moved into an endemic area

of *S. mansoni*. They showed that prevalence is related to the duration of exposure, both in the adult immigrants and in those who had been exposed to infection from early childhood. In both groups, prevalence begins to fall about 20 years after the first exposure and is not related to age or water usage of the individual.

Somewhat similar findings were reported by Omer & Amin (1973). They studied canal cleaners in the Gezira irrigated area of the Sudan. These cleaners spend up to 6 hr a day clearing weeds from canals containing infected *Biomphalaria*. Apparently, those who were new to the job often fell ill, were unable to continue work and had extremely high egg excretion rates. Those who had worked in the canals for over 5 years were much fitter and their egg counts were very low.

In both these observations, a decline in infection appears to have taken place in circumstances where exposure to infection had not altered with the passage of years.

These are not examples of definitive studies on human schistosome immunity, but they illustrate the kind of longitudinal epidemiological study which has to be done if we are to improve our knowledge of immunity in man. Until we know the situation in man, it is impossible to judge the feasibility of controlling the disease by immunological methods. If the schistosome fails to stimulate protective immunity in man, then indeed we have a very difficult task ahead. An effective vaccine will be very difficult to devise where there is no underlying natural acquired immunity.

If man develops immunity to schistosomes, even though it is an immunity that is masked by the continuing presence of the adult worms, then our task will be an easier one.

LITERATURE CITED

Butterworth, A.E., Sturrock, R.F., Houba, V. & Rees, P.M., 1974. Antibody dependent cell-mediated damage to schistosomula in vitro. Nature, Lond. 252: 503-505.

Butterworth, A.E., Sturrock, R.F., Houba, V., Mahmoud, A.A., Sher, A. & Rees, P.M., 1975. Eosinophils as mediators of antibody dependent damage to schistosomula. Nature, Lond. 256: 727-729.

Capron, A., Dessaint, J.P., Capron, M. & Bazin, H., 1975. Specific IgE antibodies in immune adherence of normal macrophages to Schistosoma mansoni. Nature, Lond. 253: 474-475.

Clegg, J.A., 1974. Host antigens and the immune response in schistosomiasis. In «Parasites in the Immunized Host: Mechanisms of Survival». Ciba Foundation Symposium 25 (new series) pp. 161-183.

Clegg, J.A. & Smithers, S.R., 1972. The effects of immune rhesus monkey serum on schistosomula of Schistosoma mansoni during cultivation in vitro. Int. J. Parasit. 2: 79-98.

Dean, D.A., Wistar, R. & Murrell, K.D., 1974. Combined in vitro effects of rat antibody and neutrophilic leukocytes on schistosomula of Schistosoma mansoni. Amer. J. trop. Med. Hyg. 23: 420-428.

Hockley, D.J. & McLaren, D.J., 1973. Schistosoma mansoni: changes in the outer membrane of the tegument during development from cercariae to adult worm. Int. J. Parasit. 3: 13-25.

Kloetzel, K. & Rodrigues Da Silva, J., 1967. Schistosomiasis mansoni acquired in adulthood: behaviour of egg counts and the intradermal test. Amer. J. trop. Med. Hyg. 16: 167.

Kusel, J.R., Sher, A., Perez, H., Clegg, J.A. & Smithers, S.R., 1975. The use of radioactive isotopes in the study of specific schistosome membrane antigens. In «Nuclear techniques in helminthology research» IV, pp. 127-143. Int. Atomic Energy Agency, Vienna.

Mahmoud, A.A.F., Warren, K.S. & Peters, P.A., 1975. A role for the eosinophil in acquired resistance to Schistosoma mansoni infections as determined by anti-eosinophil serum. J. exp. Med. 142: 805-813.

Omer, A.H.S. & Amin, M.A., 1973. Evidence of the development in man of acquired resistance in infections with Schistosoma mansoni. 9th Int. Congr. Trop. Med and Malaria, Athens II, p. 88 (Abstract).

Perez, H. & Terry, R.J., 1973. The killing of adult Schistosoma mansoni in vitro in the presence of antisera to host antigenic determinants and peritoneal cells. Int. J. Parasit. 3: 499-503.

Phillips, S.M., Reid, W.A., Bruce, J.I., Hedlund, K., Colvin, R.C., Campbell, R., Diggs, C.L. & Sadun, E.H., 1975. The cellular and humoral immune response to Schistosoma mansoni infections in inbred rats. I. Mechanisms during initial exposure. Cell. Immunol. 19: 99-116.

Sher, F.A., Mackenzie, P. & Smithers, S.R., 1974a. Decreased recovery of invading parasites from the lungs as a parameter of acquired immunity to schistosomiasis in the laboratory mouse. J. infect. Dis. 130: 626-633.

Sher, F.A., Kusel, J.R., Perez, H. & Clegg, J.A., 1974b. Partial isolation of a membrane antigen which induces the formation of antibodies lethal to schistosomes cultured in vitro. Clin. exp. Immunol. 18: 357-369.

Sher, F.A., Smithers, S.R. & Mackenzie, P., 1975. Passive transfer of acquired resistance to Schistosoma mansoni in laboratory mice. Parasitology 70: 347-357.

Smithers, S.R. & Terry, R.J., 1967. Resistance to experimental infection with Schistosoma mansoni in rhesus monkeys induced by the transfer of adult worms. Trans. roy. Soc. trop. Med. Hyg. 61: 517-533.

Smithers, S.R. & Terry, R.J., 1969. Immunity in schistosomiasis. Ann. N.Y. Acad. Sci. 160: 826-840.

Smithers, S.R. & Terry, R.J., 1976. The immunology of schistosomiasis. In «Advances in Parasitology» (Ed. Ben Dawes), 14, 399-422. Academic Press, London & New York.

EXPERIMENTAL VACCINES IN SCHISTOSOMIASIS[1,2,3]

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Anti-schistosomal vaccines are, at present, little more than a hope and an idea, and work in this area is truly in the experimental stages. While considerable work and some progress have occurred, there does not exist at this time any vaccine that could qualify for trials in humans. To facilitate discussion of the possible approaches towards a schistosome vaccine, we think it would be helpful to review the nature of the vaccines in use today for a wide variety of other infectious diseases. Hopefully, we will benefit by considering the nature of such vaccines in terms of selecting approaches to a schistosome vaccine.

As seen in Table 1, vaccines for microbial diseases consist of three basic types : antigen extracts (especially in bacterial diseases), killed whole organisms, and attenuated-live organisms (especially in anti-viral vaccines). Not all of these vaccines are entirely satisfactory, and work continues in order to improve them. Of interest to schistosome immunologists is the fact that the im-mune mechanisms responsible for protection were, and in many cases are still, not well defined ; most of these vaccines were obtained by empirical methods.

When we review the work in vaccine development in parasitic diseases (Table 2), one is immediately struck by the fact that the majority are live organism-type vaccines. Some of these agents are already in commercial production, while others are only in varying stages of experimentation and development.

When we examine the work on anti-schistosomal vaccines, the use of live organisms is also prominent among the various experimental immunogens. Live parasite-type immunizations have taken two forms : avirulent, zoophilic or heterologous schistosome species or strains, and radiation-attenuated homologous species. The third approach, immunization with crude or purified antigens, represents an approach with perhaps the widest appeal among people in this field, but is one which, unfortunately, has provided the smallest degree of success.

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TABLE 1. Nature of immunogens in vaccines for infectious disease (non-parasitic)

Disease	Immunogen
1. EXTRACTS	
Cholera	Crude Fraction of Vibrios
Plague	Crude Fraction of Bacilli
Diptheria	Purified Toxoid
Tetanus	Purified Toxoid
Meningitis	Purified Polysaccharide
Pneumonia	Purified Polysaccharide
2. KILLED ORGANISMS	
Rabies	Inactivated Virus
Influenza	Inactivated Virus
Typhus Fever	Killed Rickettsia
Thyphoid Fever	Killed Bacteria
Polio	Inactivated Virus
3. ATTENUATED ORGANISMS	
Yellow Fever	Infectious (Attenuated) Virus
Measles	Infectious (Attenuated) Virus
Mumps	Infectious (Attenuated) Virus
Rubella	Infectious (Attenuated) Virus
Polio	Infectious (Attenuated) Virus
Smallpox	Infectious (Attenuated) Virus
Tuberculosis	Infectious (Attenuated) Mycobacteria

TABLE 2. Nature of immunogens in vaccines for parasitic diseases

Disease	Immunogen
Coccidiosis	Attenuated Oocysts
Hookworm (Dog)	Attenuated Larvae
Lung Worm (Cattle)	Attenuated Larvae
— "Very Experimental" —	
Malaria	Attenuated Sporozoite
Taeniasis (Sheep)	Normal Larvae in Abnormal Site
Filariasis (Various)	Attenuated Larvae
Trichinosis	Larval Extract

Before presenting some of our work on the use of heterologous cercariae as an immunizing agent, we would like to review quickly some of the work of others using this approach. As you can see (Table 3), not always consistent results have been obtained between different groups of investigators, particularly in experiments with monkeys. Taylor and his associates (1973) attempted a novel scheme whereby F_1 hybrid cercariae from the mating of *Schistosoma rodhaini* and *S. bovis* were used to immunize baboons against *S. mansoni* challenge. The results, however, were not encouraging. In the majority of these cases, the levels of immunity based on adult worm recoveries were moderate. In contrast, in rhesus monkeys immunized with the zoophilic strain of *S. japonicum,* egg excretion data indicated a very high level of induced resistance. This prompted our experiment (Murrell et al., 1973). Briefly, monkeys were exposed five times to about 1,700 cercariae of the zoophilic Formosan strain of *S. japonicum* and then challenged with the lethal Philippine strain. At autopsy 6 weeks later, 52% fewer challenge worms survived in the immunized monkeys (Table 4). Likewise, immunized monkeys shed far fewer eggs. When liver tissue egg counts were compared, however, no difference was noted. Since the egg is the primary agent of the disease, the level of immunity induced from a clinical standpoint was not satisfactory. The organ pathology was, as you would expect, considerable in both groups. We were further discouraged from pursuing this vaccination approach by the high degree of difficulty and impracticality in generating large numbers of immunizing cercariae where and when they were needed. These results do not, however, prove the unfeasibility of this particular method, since the zoophilic strain might be sufficiently immunogenic in humans to prevent the accumulation of unacceptable numbers of eggs. But it would be extremely difficult, based on these studies and those of others, to justify human experimentation, especially with *S. japonicum.*

In contrast, the work now going on in the laboratory of Taylor and Nelson at Winches Farm in London, using irradiated cercariae, appears to offer a more hopeful approach. These investigators have, with such an experimental vaccine, developed a truly effective procedure in large domestic animals. In Table 5, many of the earlier studies on the immunogenicity of irradiated cercariae are shown. Consistent, although moderate, levels of immunity were induced in rodents with 2-3 kilorads. Some discrepancy has appeared in the case of monkeys, however. Attenuating conditions in different laboratories, as well as variations in immunization protocols and numbers of cercariae used, may help to explain some of these contradictions. As already mentioned, the group at Winches Farm in London has had very good results in sheep. Dr. Taylor will be presenting their recent work at a later session which should be of great interest to all concerned with vaccines.

Our work along this line has produced some uncertainties regarding the ease of standardization of protocols for immunizing with irradiated cercariae. In experiments in mice (Table 6), we were not able to induce significant levels of immunity with the irradiation doses utilized by others, usually 2-3 kilorads. When the dosage was increased to 8-16 kilorads, immunity *was* obtained. Importantly, no immunizing worms survived at these irradiation levels. Similar findings have been reported by Taylor in sheep. At this time we can only speculate that the discrepancy between our effec-

TABLE 3. Selected studies on heterologous immunity in Schistosomiasis

Host	Immunizing species or strain	Challenge Species	Protection	Authors
Rhesus Monkey .	*Schistosomatium douthitti*	*Schistosoma mansoni*	(—)	Kagan (1953)
Rhesus Monkey .	*Schistosomatium douthitti*	*S. japonicum*	(+)	Hsü et al. (1964)
Rhesus Monkey .	*S. japonicum* (Formosan)	*S. japonicum*	(+)	Hsü and Hsü (1963)
Baboons	*S. rodhaini*	*S. mansoni*	(+)	Taylor et al. (1973)
Baboons	*S. bovis*	*S. mansoni*	(+)	Taylor et al. (1973)
Baboons	Hybrids	*S. mansoni*	(+)	Taylor et al. (1973)
Sheep	*S. mansoni*	*S. mattheei*	(—)	Taylor (1975)

TABLE 4. Immunization of Rhesus monkeys with the zoophilic Formosan strain of *Schistosoma japonicum* (Murrell et al., 1973)

Measurement	Immunized	Controls	% Difference
Adult Worm (Recovery ($\overline{X} \pm SE$)	81 ± 26	167 ± 15	52
Mean Eggs /g Stool ($\overline{X} \pm SE$)	22 ± 6	95 ± 17	77
Mean Egg /g Stool/Female Worm	0.55 ± 0.16	1.40 ± 0.15	61
No. Eggs/10g Liver Tissue	75,375	71,700	0

TABLE 5. Studies on immunization against schistosomes with irradiated cercariae

Host	Schistosome Species	Radiation	Protection	Authors
Mice	*S. mansoni*	^{60}Co	(+)	Villella (1961)
Mice	*S. mansoni*	^{60}Co	(+)	Radke & Sadun (1963)
Mice	*S. mansoni*	X-ray	(+)	Perlowagora-Szumlewicz (1964)
Mice and Rats . .	*S. mansoni*	^{60}Co	(+)	Erickson & Caldwell (1965)
Rats	*S. mansoni*	X-ray	(+)	Smithers & Terry (1965)
Rhesus Monkey . .	*S. mansoni*	^{60}Co (2.5k)	(+)	Sadun et al. (1964)
Rhesus Monkey . .	*S. mansoni*	^{60}Co (4.0k)	(—)	Sadun et al. (1964)
Rhesus Monkey . .	*S. mansoni*	X-ray (2.0k)	(—)	Smithers & Terry (1967)
Rhesus Monkey . .	*S. japonicum*	X-ray (24k, 48k)	(+)	Hsü et al. (1969)
Sheep	*S. mattheei*	^{06}Co	(+)	Taylor (1975)

tive irradiation levels and those reported by others may lie in differences in strains of parasite and host. This experience should serve as a warning that different conditions may call for alterations in published protocols, in particularly with respect to minimum attenuating irradiation dose.

We have also investigated another possible source of irradiation for cercarial attentuation with the intent to make irradiation more adaptable to field-type conditions. As you know, gamma or x-ray irradiation is not easily performed in the field. As such, we have experimented with ultraviolet light, which has the advantages that it is relatively safe and can be generated with simple and easily available equipment.

Our experiments have as yet dealt only with mice, but as you can see in Table 7, we were able to achieve moderate to high levels of protection ; however, we have not yet induced immunity in the complete absence of residual immunization worms.

I wish to emphasize again that, although irradiated cercariae show great promise in relation to other vaccine approaches, their practicality is subject to debate. Because cercariae have such a short shelf life, on the order of hours, eventual wide application of the procedure, even as a domestic animal vaccine, will require the maintenance, under field conditions, of large snail-cercariae factories and sophisticated irradiation sources. This does not promise to be an inexpensive and simple task. However, a solution to this may be near. In an imaginative series of studies, James, Farrant, & Taylor (pers. comm., 1975), of the London School of Hygiene and Tropical Medicine, have shown that irradiated cercariae in large numbers can be easily transformed *in vitro* to the schistosomule stage, and injected by needle into the host, producing immune levels comparable to that achieved with cercariae. Going further, they have been successful in freezing schistosomules down to —20°C and recovering full infective organisms on thawing. If schistosomules can be cryopreserved, many of the practical drawbacks to the development of an irradiated cercarial vaccine can be corrected. Whatever the eventual outcome, the information to be gained by experimentation with this approach will be of great value to those interested in the immunology of schistosomiasis.

TABLE 6. Resistance induced in NIH/NMRI* mice with two exposures to 500 ^{60}Co-irradiated *Schistosoma mansoni* cercariae

Level of Irradiation (Kr)	Reduction in Challenge Worm Survival (%)
2.5	0
4.0	29
8.0	49
16.0	56

(*) National Institutes of Health/Naval Medical Research Institute inbred strain of white Swiss mice.

TABLE 7. Resistance induced in mice exposed to *Schistosoma mansoni* cercariae irradiated with ultra-violet light (2537 Å)

Number Immunizations	Number Immunizing Cercariae	Reduction in Challenge worm survival (%)
1	50	0
1	100	1
1	250	41
1	500	66
2	500	93
2	500	55

I would like to turn now to the third basic type of vaccine, that of antigen extracts. This approach has always had great appeal to immunoparasitologists for both its potential practicality and the assumed reduced hazards in its use. As reflected in Tables 8 and 9, the results have been less than promising inspite of considerable efforts toward this goal. Immunization with whole eggs, or cercariae, or with their extracts, has failed to induce protection in all but two studies of which we are aware. An exciting report was made 6 years ago by Dodin (1969).

That author claimed to have reduced substantially the reinfection rate in Ambilhar-treated children by immunization with lyophilized cercariae previously treated with ascorbic acid and copper. To my knowledge, though, there has not been a published follow-up of this work.

The effectiveness of adult worm extracts has had more claims ; about two-thirds of published studies reported success in inducing protection, primarily in mice. It must be pointed out, however, that the levels of immunity induced were usually marginal.

TABLE 8. Immunization with inactivated egg and cercarial stages or their antigenic extracts

Immunogen	Protection	Authors
1. Egg Extracts	—	Kagan (1958)
		Smithers (1962)
		Ritchie et al. (1962)
2. Whole Cercariae	—	Thompson (1954)
Whole Cercariae treated with ascorbic acid and copper	+	Dodin (1969)
3. Cercarial Extracts	+	Ozawa (1930)
	—	Sadun & Lin (1959)
	—	Ritchie et al. (1962)

TABLE 9. Immunization with adult worm antigens

Immunogen	Resistance to Challenge	Authors
1. Adult Worm Extracts	+	Ozawa (1930)
Adult Worm Extracts	+	Kawamura (1932)
Adult Worm Extracts	+	Watts (1949)
Adult Worm Extracts	—	Vogel & Minning (1953)
Adult Worm Extracts	+	Sadun & Lin (1959)
Adult Worm Extracts	+	Sadun & Bruce (1964)
Adult Worm Extracts	—	Kagan (1958)
Adult Worm Extracts	—	Ritchie et al. (1962)
Adult Worm Extracts and BCG	+	Capron & Lesoin (1969)
2. Adult Culture Antigens	+	Sadun & Lin (1959)
Adult Culture Antigens	+(?)	Murrell & Clay (1972)
3. Adult and Cercarial Culture Antigens (Mixed)	(+)	Levine & Kagan (1960)

In our own study on adult culture antigen, we are compelled to say that, although a moderate level of protection was induced in the first trial, we were not able to confirm that result in two subsequent attempts. The highest levels of immunity induced by antigen extracts were those reported by Capron & Lesoin (1969) who used BCG (Bacille Calmette Guérin) as an adjuvant. However, these workers have not had consistent success in repeat experiments with this procedure which, unfortunately, seems to be true for most of these studies. To illustrate further the frustration experienced by ourselves, and perhaps by those others

struggling with this problem, we have summarized our experimentation (Murrell et al., 1975) on antigen extracts made in a variety of ways in Table 10. As you can see, we consistently failed to produce immunity with crude adult worm extracts, regardless of the adjuvant employed, except in an initial experiment designed to recover surface-associated antigens using 3 molar potassium chloride (3M KCl). Frustratingly, we have not been able to confirm those initial results. Again, with cercarial autolytic antigen and, as mentioned, with adult culture antigen, initial success was followed with repeated failure.

TABLE 10.   Immunization with *S. mansoni* antigen fractions
(Murrell, Dean and Stafford, 1975)

Immunogen	Animal	Cytotoxic Antibody	% Reduction in Challenge worm Survival
1. Adult Worm Crude Extracts			
FCA	Mice	+	0
ICFA	Mice	+	0
Adjuvant*			
Alum & *Bordatella*			
pertussis	Mice	+	0
BCG	Mice	+	0
FCA	Guinea Pigs	+	0
2. Hypertonic Salt Extract (3M KCl)			
1	Mice	+	27
2	Mice	+	1
3	Guinea Pigs	+	0
3. Cercarial Enzyme Secretions . . .	Mice	±	0
4. Cercarial Autolytic Antigen			
1	Mice	+	48
2	Mice	+	0
3	Mice	0	15
5. Adult Culture Antigen			
1	Mice	0	47
2	Mice	0	0
3	Rats	0	0

*FCA = Freund's complete adjuvant
IFCA = Incomplete Freund's adjuvant
BCG = Bacille Calmette Guérin

These results should not, however, deter anyone from undertaking the challenge of an antigen-extract vaccine. Rather, we hope they will serve to stimulate reflection on the problems associated with complex antigen extracts on which relatively little is known of their chemical and immunological nature. This is emphasized in Table 11 in which we have attempted to outline some major problems in this field that may account for the wide variations in results obtained by different groups of investigators.

Although it was stated in the beginning that most anti-microbial vaccines have come about by essentially empirical means, it is only fair to point out that, in contrast to human schistosomiasis, microbial diseases often induce very high levels of resistance, indicating that their antigens are immunogenically of a high order. In schistosomiasis, the levels of immunity, as a result of primary infection, are not always high and, in the case of humans, they may be quite moderate. A solution to the problem of low immunogenicity of schistosome antigens may lie in the manipulation and modification of antigens in such a manner as to elicit immune responses of a greater effectiveness than would result from normal infection. This undertaking, of course, will require detailed immunochemical knowledge of the antigens. At the same time, a precise understanding of the mechanism of immune resistance would be of immeasurable help in reaching that goal.

Towards that end, we are studying in our laboratory the role of humoral antibody in protection. I would like to present *one* current experiment which I think will illustrate the potential contributions that an understanding of the immune mechanisms can make towards vaccine development. In this experiment, we attempted to evaluate the role of reagins, or homocytotropic antibody, in immune protection. Ishizake et al. (1957) have suggested that reaginic antibody may have an important role in infectious diseases by promoting, through its induction of vascular permeability, the translocation of increased amounts of antibody and cells into the infected tissues. This has been adapted by us in a hypothesis illustrated in Fig. 1.

We tested the hypotheses by implanting intradermally (i.d.) in the abdominal skin of mice, mouse sera with a high titer of reagin. The mice were also injected intravenously (i.v.) with serum from immune mice having a chronic infection. Seventy-two hours after the implantation of reagin, the mice were challenged by allowing 100 cercariae to penetrate

TABLE 11. **Difficulties encountered in reproducing immunization results obtained by different investigators**

Some of these difficulties may be due to the:

1. Use of different schistosome and host strains;
2. Inadequate standardization of methods and protocols, particularly in preparing antigens and in assessing host resistance;
3. Faulty experimental designs resulting in inadequate numbers of test animals and insufficient controls; and
4. Scarce immunochemical information on schistosome antigens, particularly with regard to antigen susceptibility to enzymes and to changes in physical-chemical conditions.
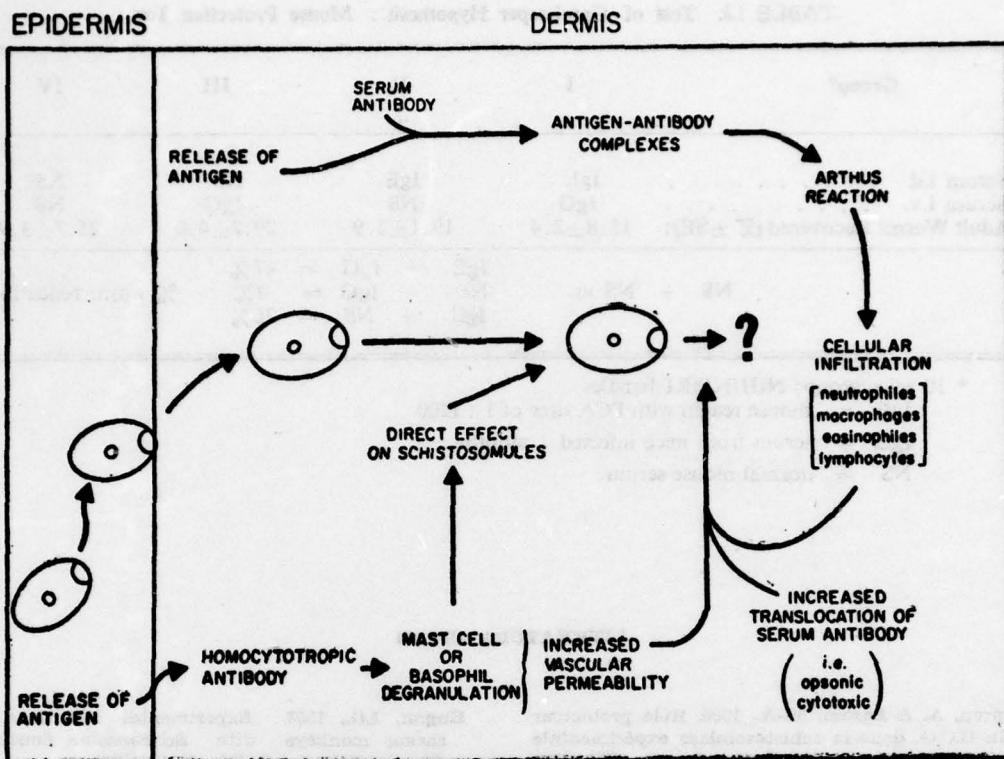
Fig. 1. The migration of the schistosomule through the dermis may, through the elaboration of antigens, trigger an immediate hypersensitivity reaction; this reaction may in turn promote the translocation of antibodies and killer cells into the tissue site. This sequence of interrelated immune reactions is the basis of of the "Gatekeeper Hypothesis."

through the serum-infiltrated skin site. Table 12 presents the results of that experiment. Mice receiving both immune serum i.v. and reagin i.d. had significantly fewer surviving challenge worms than the controls. However, these two antibodies, in combination with normal mouse serum, failed to protect mice.

These results in themselves do not prove the indispensable role of reagin and immune IgG and will require confirmation, but they do encourage us in the belief that it may be possible to induce such protective immune responses by selecting from the large array of schistosome antigens those capable of inducing effective reagin and effective IgG responses. Such knowledge would permit

focusing on these antigens attempts to bolster their immunogenicity. Regardless of the eventual outcome of this particular work, the approach serves as an example of how defined immune mechanisms can further our search for a vaccine.

In summary, we feel that there do exist grounds for optimism that an immunological control of schistosomiasis can be developed; the attenuated-live cercariae approach certainly warrants continued support and interest. At the same time, encouragement must be maintained for those attempting to unravel the complex immune response to schistosomiasis, for solutions to this problem will depend on a precise and complete understanding of this dangerous and successful parasite.

TABLE 12. Test of Gatekeeper Hypothesis : Mouse Protection Test

Group*	I	II	III	IV
Serum i.d	IgE	IgE	NS	NS
Serum i.v.	IgG	NS	IgG	NS
Adult Worms Recovered ($\overline{X} \pm SE$):	13.8±2.4	19.1±1.9	29.2±4.0	25.7±3.9

$$NS + NS \; vs. \quad \begin{array}{l} IgE + IgG = 47\% \\ Ns + IgG = 0\% \\ IgE + NS = 26\% \end{array} \quad \% \text{ worm reduction}$$

* 10 mice/group; NIH/NMRI females
    IgE = mouse reagin with PCA titer of 1 : 1200
    IgG = serum from mice infected 7 months
    NS = normal mouse serum

LITERATURE CITED

Capron, A. & Lesoin, M.-A., 1969. Rôle protecteur du B.C.G. dans la schistosomiase expérimentale à S. mansoni. C.R. Acad. Sci. Ser. D. 269: 2110-2112.

Dodin, A., 1969. Immunological changes during treatment with Niridazole: Practical applications of protection from reinfection in an endemic zone. Ann. N.Y. Acad. Sci. 160: 880-884.

Erickson, D.G. & Caldwell, W.L., 1965. Acquired resistance in mice and rats after exposure to gamma-irradiated cercariae. Amer. J. trop. Med. Hyg. 14: 566-573.

Hsü, H.F., Hsü, S.Y. Li & Tsai, C.T., 1964. Immunization against Schistosoma japonicum in rhesus monkeys by administration of cercariae of Schistosomatium douthitti. Z. Tropenmed. Parasit. 15: 435-440.

Hsü, S.Y. Li & Hsü, H.F., 1963. Further studies on rhesus monkeys immunized against Schistosoma japonicum by administration of cercariae of the Formosan strain. Z. Tropenmed. Parasit. 14: 506-512.

Hsü, S.Y. Li, Hsü, H.F. & Osborne, J.W., 1969. Immunization of rhesus monkeys against schistosome infection by cercariae exposed to high doses of x-radiation. Proc. Soc. Exp. Biol. Med. 131: 1146-1149.

Ishizaka, K., Ishizaka, T., Sugaharu, T. & Maruyama, S., 1957. Studies on the relationship between toxin anaphylaxis and antitoxic immunity. III. Significance of allergic action on the neutralization of toxin injected intracutaneously. Jap. J. med. Sci. Biol. 10: 343-361.

Kagan, I.G., 1953. Experimental infections of rhesus monkeys with Schistosoma douthitti (Cort, 1914). J. inf. Dis. 93: 200-206.

Kagan, I.G., 1958. Contributions to the immunology and serology of schistosomiasis. Rice Inst. Pamphl. 45: 151-183.

Kawamura, R., 1932. The recent researches on schistosomiasis in Japan. Compt. Rend. Congr. Int. Med. Trop. Hyg. (Cairo, 1928) 4: 311-319.

Levine, D.M. & Kagan, I.G., 1960. Studies on the immunology of schistosomiasis by vaccinations and by passive transfer. J. Parasit. 46: 787-792.

Murrell, K.D. & Clay, B., 1972. In vitro detection of cytotoxic antibodies to Schistosoma mansoni schistosomules. Amer. J. trop. Med. Hyg. 21: 569-577.

Murrell, K.D., Dean, D.A. & Stafford E.E., 1975. Resistance to infection with Schistosoma mansoni after immunization with worm extracts or live cercariae: Role of cytotoxic antibody in mice and guinea pigs. Amer. J. trop. Med. Hyg. 24: 955-962.

Murrell, K.D., Yogore, M.G., Jr., Lewert, R.M., Clutter, W.G. & Vannier, W.E., 1973. Immunization with a zoophilic strain of Schistosoma japonicum: A re-evaluation of the Formosan strain of S. japonicum in rhesus monkeys. Amer. J. trop. Med. Hyg. 22: 723-733.

Ozawa, M., 1930. Experimental study on acquired immunity to Schistosomiasis japonica. Jap. J. exp. Med. 8: 79-84.

Perlowagora-Szumlewicz, A., 1964. Studies on acquired resistance to Schistosoma mansoni in mice exposed to x-irradiated cercariae. Bull. Wld Hlth Org. 30: 401-412.

Radke, M.G. & Sadun, E.H., 1963. Resistance produced in mice by exposure to irradiated Schistosoma mansoni cercariae. Exp. Parasit. 13: 134-142.

Ritchie, L.S., Garson, S. & Erickson, D.C., 1962. Attempts to induce resistance against Schistosoma mansoni by injecting cercarial, adult worm, and egg homogenates in sequence. J. Parasit. 48: 233-236.

Sadun, E.H. & Bruce, J.I., 1964. Resistance induced in rats by previous exposure to and by vaccination with fresh homogenates of Schistosoma mansoni. Exp. Parasit. 15: 32-43.

Sadun, E.H., Bruce, J.I. & Macomber, P.B., 1964. Parasitologic, pathologic and serologic reactions to Schistosoma mansoni in monkeys exposed to irradiated cercariae. Amer. J. trop. Med. Hyg. 13: 548-557.

Sadun, E.H. & Lin, S.S., 1959. Studies on the host-parasite relationship to Schistosoma japonicum. IV. Resistance acquired by infection, by vaccination, and by the injection of immune serum in monkeys, rabbits and mice. J. Parasit. 45: 543-548.

Smithers, S.R., 1962. Stimulation of acquired resistance to Schistosoma mansoni in monkeys: Role of eggs and worms. Exp. Parasit. 12: 263-273.

Smithers, S.R. & Terry, R.J., 1965. Acquired resistance to experimental infections of Schistosoma mansoni in the albino rat. Parasitology 55: 711-717.

Smithers, S.R. & Terry, R.J., 1967. Resistance to experimental infection with Schistosoma mansoni in rhesus monkeys induced by the transfer of adult worms. Trans. roy. Soc. trop. Med. Hyg. 61: 517-533.

Taylor, M.G., 1975. Towards the development of a live vaccine for schistosomiasis. Int. Atom. Energ. Agency, Vienna. pp. 165-173.

Taylor, M.G., Nelson, G.S., Smith, M. & Andrews, B.J., 1973. Studies on heterologous immunity in schistosomiasis. Bull. Wld Hlth Org. 49: 57-65.

Thompson, J.H., Jr., 1954. Host-parasite relationships of Schistosoma mansoni. Exp. Parasit. 3: 140-160.

Villella, J.B., Gomberg, H.J. & Gould, S.E., 1961. Immunization to Schistosoma mansoni in mice inoculated with radiated cercariae. Science 134: 1073-1074.

Vogel, H. & Minning, W., 1953. Ueber die erworbene Resistenz von Macacus rhesus gegenüber Schistosoma japonicum. Z. Tropenmed. Parasit. 4: 418-505.

Watts, N.P., 1949. Prophylactic use of schistosomal antigen. J. Immunol. 62: 183-192.

RECENT ADVANCES IN THE DIAGNOSIS OF SCHISTOSOMIASIS

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By evaluating studies made on serologic procedures used for diagnosing schistosomiasis in general population surveys, we may highlight some of the problems involved in the use of these tests. After making a survey in Ethiopia, Buck et al. (1964) ranked the complement-fixation (CF) test, skin test (ST), flocculation tests and immunofluorescent (IF) test in this order of sensitivity, for the diagnosis of schistosomiasis in children 1-9 years of age who were passing eggs of *Schistosoma mansoni*. In a study made in Uganda in areas of high and low endemicity for schistosomiasis, Moriearty & Lewert (1974) evaluated the immediate (IHT) and the delayed skin reaction (DHT) tests and compared these tests with serologic tests. In this study the circumoval precipitin test (COP), double diffusion (DD), CF, and IF tests were compared in persons passing schistosome eggs. The IF and COP were most sensitive, followed by the CF and the DD technique. In a study made on St. Lucia in the Caribbean area in a population infected with *S. mansoni*, Warren et al. (1973) compared the intradermal test with five antigen and three serologic tests. Using an adult worm antigen, they noted DHT reactions in 66% of the individuals tested. This contrasted with 29% positive reactors observed in Uganda by Moriearty & Lewert (1974). Both of these groups used the identical antigen made at the Center for Disease Control. Warren et al. (1973) evaluated the CF, cholesterol-lecithin (CL), and IF tests, and the only test that gave acceptable levels of sensitivity was the IF test. Both the CF and CL tests gave too many false negatives, and the CF gave too many false positives with sera from persons on an adjacent island where schistosomiasis is not being transmitted.

Several serologic tests were evaluated in Rhodesia with sera from persons infected with either *S. mansoni* or *S. hematobium,* and the sensitivity of the three flocculation tests [bentonite (BFT), CL, and a charcoal flocculation (ChF) test] was compared with that of the CF, IF, and indirect hemagglutination (IHA) tests. The sensitivity of the flocculation tests varied from 55 to 68% : 48% for CF, 57% for IF, and 47% for IHA (Allain et al., 1972). The same antigens and tests were found more sensitive when sera from patients with clinical schistosomiasis were tested at the Center for Disease Control.

It is thus evident that one cannot generalize about the sensitivity and specificity of a particular serologic test. The IF test proved to be nonspecific in Ethiopia and acceptable in Rhodesia and St. Lucia. The CF test was most sensitive in Ethiopia, insensitive in Uganda, and sensitive but nonspecific in St. Lucia. It ap-

pears that a number of factors such as age of the individual, intensity of exposure to schistosome cercariae, immune status of the individual, plus technical factors such as quality of the antigen used and interlaboratory variation for each particular technique may play a role in the reactivity of serologic tests that are antibody dependent. It is also apparent that detecting antibodies is much more difficult in asymptomatic individuals than in clinically ill-patients. It is for this reason that more than one serologic technique has been recommended for evaluation of the prevalence of schistosome antibody (McCarten et al., 1975). Under ideal circumstances, a pilot study should be made to evaluate this point. If this is not possible, the most sensitive and specific test for an area under study must be carefully selected by reviewing the published literature.

The diagnosis of schistosomiasis in the clinically ill-patient is readily made. Practically any of the 15 or more serologic tests that have been used in the diagnosis of schistosomiasis provide adequate diagnostic sensitivity. The specificity of the serologic tests is a problem since some tests cross-react with other trematode infections. In areas of the world where fascioliasis, clonorchiasis, and paragonimiasis are a problem, tests of high specificity are preferred. In the Orient, the COP test appears to be the serologic test of choice. I believe this test has been selected because of its technical simplicity coupled with high sensitivity and specificity.

The diagnosis of the lightly infected asymptomatic person passing schistosome eggs presents a greater challenge with regard to sensitivity and specificity. This is especially true for children and young adults. For these persons, the *in vitro* tests with cercariae (Zercarien-Hüllen

Reaktion) and the COP appear to be very useful. Tests of high sensitivity generally have decreased specificity because the number of false positive reactions increases. To overcome this problem, a number of new serologic techniques have been developed which may prove to be very useful in future studies.

One of the most sensitive and specific techniques for the serologic diagnosis of schistosomiasis is the IF test. An important variable in the use of the IF test has been the character and nature of the antigen. Cercariae were initially used by Sadun et al. (1960) as antigen in the test. However, antigen nonspecificity, perhaps due to contact with nonhuman schistosomes, resulted in unacceptable numbers of false positive reactions; to overcome this problem, frozen sections of adult worms have been introduced as the antigen of choice (Wilson et al., 1974). Other attempts to overcome the antigen problem have led to the development of techniques that utilize cellulose acetate discs impregnated with soluble schistosome antigen. The SAFA (soluble antigen fluorescent antibody) test (Toussaint & Anderson, 1965) is such a technique. The mechanical problem of handling the discs and reading the reactions with a fluorometer has prevented wide acceptance of this method (Sadun & Gore, 1967 ; Deelder, 1973).

A technique which is an adaptation of the radioimmunoassay uses the binding of ^{125}I to schistosome antigen. This radioactive antigen microprecipitin (RAMP) assay has not gained wide acceptance because it is difficult to perform and requires highly specialized equipment (Williams et al., 1971).

In 1974 a quantitative immunofluorescent technique called DASS system (defined antigen substrate spheres) was introduced by Deelder & Ploem (1974) for

the diagnosis of schistosomiasis. This technique is based on the principle that Sepharose coupled to antigen can be used as a substrate in the IF test. The intensity of the fluorescence is quantitated with a photomultiplier connected to a fluorescence microscope and is correlated with the amount of antibody present in the sample. The fluorescence is expressed in arbitrary units as the relative fluorescein intensity.

Sepharose beads are pure agarose beads which come in several forms depending on the percentage of agarose used in preparation (2%, 4%, and 6%). In the schistosomal system, 1 mg of schistosome antigen is coupled to 1 ml of 4B Sepharose beads which have been activated by 20-50 mg of CNBr and deactivated after coupling with 4-amino-n-bulyric acid. The test is performed with 5-10 μl of bead suspension diluted in serum in phosphate buffer (PBS) that contains 4% ovalbumin. The beads can be stored in a 0.02% NAN$_3$ solution in PBS, or freeze-dried in a 2.5% lactose-dextran solution (Deelder & Ploem, 1974). Although this technique is very promising, the necessity for a photomultiplier and a fluorescence microscope has deterred wide acceptance. The DASS system may also be used to measure antigen by coupling antibody to the beads. The main technical aspects of the technique are outlined by Capel (1975).

A second newly developed procedure is an enzyme-linked assay called ELISA (enzyme-linked immunosorbent assay). In this modification of the IF technique, horse - radish - peroxidase or alkaline phosphatase enzyme is substituted for the fluorescein isothiocyanate in the anti-immunoglobulin conjugate. After the interaction of antigen with serum with the enzyme conjugate, a suitable substrate is added which causes a color reac-

tion of the enzyme present in the antigen-antibody complex. The amount of antibody in the test serum thus is directly related to the intensity of the color reaction. The color change, depending on the particular technique, can be measured by a colorimeter or by the eye, and the method lends itself readily to automation. In one method, specific antibodies can be measured by enzyme-labelled anti-immunoglobulin in antigen-coated polystyrene tubes. The technique is thus very similar to radioimmunoassay for the detection of antigens and antibody. A measure of the efficacy of the technique is indicated by its widespread study in parasitic diseases. The assay has been applied for the detection of antibody in persons with *Trichinella spiralis* infection (Ljungström et al., 1974 ; Ruitenberg et al., 1974, 1975b), malaria (Voller et al., 1974), Chagas' disease (Ferreira et al., 1975), toxoplasmosis (Ourth et al., 1974), and schistosomiasis (Ferreira et al., 1974 ; Huldt et al., 1975 ; Bout et al., 1975). Bout et al. (1975) have also adapted the technique for the diagnosis of hydatid disease, filariasis, amebiasis, and hookworm.

In the schistosomal ELISA test, Ferreira et al. (1974) used fragments of lyophilized adult schistosome worms as antigen, which was preserved in 1.5% formol and was fixed to slides. The horseradish-peroxidase enzyme was conjugated to the antihuman globulin by means of 1% gluteraldehyde. The enzyme substrate color reaction was developed with a solution of 5 mg of diaminobenzidine tetracyclochloride in 10 ml of a Tris-HCl buffer 0.05 M, pH 7.6, with 0.01% hydrogen peroxide. When 25 sera of persons passing eggs of *S. mansoni* were compared by ELISA, IF, and IHA, the authors found the ELISA titers to be similar or one dilution higher than the IF test and slightly lower in titer than the IHA test.

Huldt et al. (1975) used a crude extract of delipidized adult worm plus two antigens purified by affinity chromatography from adult worm and eggs of *S. mansoni*. The antigens were affixed to plastic tubes which were then washed to remove all free material. After incubation with test serum, the antigen-antibody complex alloted to this tube was washed and an alkaline phosphatase labelled conjugate was used. This in turn was activated by p-nitrophenylphosphate in 0.5 M carbonate buffer, pH 9.8, containing 0.5 M $MgCl_2$. After 15 min the reaction was stopped with 2 M NaOH, and the intensity of the color reaction was read in a colorimeter. In tests with 20 sera from patients in Zaire and 19 patients treated in Sweden (9 Swedish and 10 African) as well as 20 control sera from normal Swedes, Huldt et al. (1975) showed that the ELISA gave higher extinction values with sera from infected persons than with sera from controls. The test was sensitive and could be adapted for seroepidemiologic purposes. A rather high background color obtained with the antigen suggests that some nonspecific reactions do occur. The authors believe further study is warranted.

Bout et al. (1975) also used soluble antigen that was affixed to polystyrene tubes. Using horse-radish-peroxidase as the enzyme in their conjugate, these authors evaluated 15 schistosomal sera with 10 sera from patients with *Fasciola hepatica*, 8 with hydatid disease, 10 with filariasis, 10 with amebiasis, and 10 with hookworm plus 12 negative controls; they found that the ELISA test was 100% specific and no cross-reactions were obtained.

Ruitenberg et al. (1975a) adapted the ELISA test to microtitration plates. Ruitenberg et al. (1974) have automated the ELISA test, and Deelder & Streefkerk (1975) have adapted ELISA to the DASS test. With a peroxidase enzyme conjugated serum and Sepharose beads coupled to antigen, a field test can be made that can be read by eye. The test can also be read with an ordinary microscope if the beads are dehydrated in alcohol and xylene and mounted on a slide. If the reaction is negative, the beads are colorless, whereas if it is positive they are brown. In a comparison of DASS-ELISA to the IFA (indirect immunofluorescent antibody) test, exceedingly high titers were obtained with the former. High specificity for the reaction was also observed.

The detection of antibody has been the primary focus of serologic tests for the diagnosis of schistosomiasis. Although free antigen was reported in the urine of rabbits experimentally infected with *Schistosoma japonicum* by Okabe & Tanaka in 1958, acceptable tests for the detection of antigen in the body fluids of man have not been developed. Berggren & Weller (1967) demonstrated antigen in the plasma of mice infected with *S. mansoni* by immunoelectrophoresis (IE). The mice, however, had to be heavily infected to be positive. In 1974, Bawden & Weller were able to increase the sensitivity of antigen detection by using a CF test. Nash (1974) and Lichtenberg et al. (1974) localized the antigen in the epithelial cells of the gut wall of the adult worm. Nash et al. (1974) characterized the antigen as a polysaccharide. Madwar & Voller (1975) utilized DD and were able to detect circulating soluble antigen-antibody complexes in human sera. They were able to detect antigen in the serum of 7 of 20 sera from patients with proven current *S. haematobium* infections and they detected antibody in the serum of 13 individuals. The ELISA-DASS system for the detection of antigen may be a most sensitive method and warrants evaluation.

Gel diffusion methods are gaining wide acceptance in the serology of parasitic diseases (Kagan, 1974). Because of its speed and simplicity, countercurrent electrophoresis (CEP) has become a most attractive technique. It has been used in schistosomiasis for the detection of antigen-antibody complexes. Scapin & Tendler (1975) reported excellent sensitivity in a CEP test with eight sera from patients with schistosomiasis that were positive in double diffusion tests. In the CEP test, the reaction occurred within 35 minutes. The authors claim the test can be shortened to 10 or 15 minutes. Seitz (1975) reports a method for the simultaneous detection of antigen or antibody in a CEP test with the serum of mice experimentally infected with malaria. The tests are made on cellulose acetate membranes in a Beckman microzene cell. The technique should be evaluated for use in the detection of schistosomiasis.

The excellent studies by Moriearty & Lewert (1974) and Warren et al. (1973) on the intradermal test form a solid foundation for future studies. Warren et al. (1973) confirmed that intradermal tests were less sensitive in children and that the delayed reaction was less sensitive but more specific. They also confirmed that cross-reactions occurred in humans who have been exposed to animal schistosome cercariae.

Warren et al. (1973) reported the prevalence of delayed skin reactions to be 66% for St. Lucia, and Moriearty & Lewert (1974) reported it to be 29% for Uganda. This intriguing difference merits further study. The relationship of skin reactivity to the immunologic status of the host has not been critically studied.

Laboratory evaluation of specific cellular immunologic competency can be made by *in vitro* studies such as migration inhibition factor (Wolfson et al., 1972) and lymphocyte transformation (Oppenheim et al., 1968) in the presence of *S. mansoni* antigen. General immunologic studies on thymus (T) dependent and bursal dependent (B) cells, including investigation of skin test responses to a battery of ubiquitous antigens and of the lymphoproliferative response to nonspecific mitogens may lead to further insights into the value of these techniques in the diagnosis and study of schistosomiasis.

Finally, all diagnostic procedures rely on the purity and specificity of the antigens used. Most antigens are crude mixtures containing specific and nonspecific components. A highly purified proteolytic enzyme which gives rise to immediate type hypersensitivity in schistosome infected patients has been developed by Saver & Senft (1971). This enzyme prepared from adult worms is extracted in acid solution (pH 4.0), concentrated on an amicon P-10 membrane, and fractionated on a Sephadex G-75 column. The enzyme-containing fractions are coupled to phenyl-abanine cyanibromide Sephadex beads and are eluted by lowering the pH to 2.5. This enzyme has been evaluated in St. Lucia with a crude skin test antigen, and although it was slightly less reactive, it may have been slightly more specific (Senft & Maddison, 1975).

In summary, this paper has dealt with the problem of detecting the lightly infected asymptomatic individual in populations with schistosomiasis.

LITERATURE CITED

Allain, D.S., Chisholm, E.S. & Kagan, I.G., 1972. Use of the bentonite flocculation test for the diagnosis of schistosomiasis. Health Services Reports, 87: 550-554.

Bawden, M.P. & Weller, T.H., 1974. Schistosoma mansoni circulating antigen: Detection by complement fixation in sera from infected hamsters and mice. Amer. J. trop. Med Hyg., 23: 1077-1084.

Berggren, W.L. & Weller, T.H., 1967. Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with Schistosoma mansoni. Amer. J. trop. Med. Hyg., 16: 606-612.

Bout, D., Dugimont, J.C., Farag, H. & Capron, A., 1975. Le diagnostic immunoenzymologique des affections parasitaires. II. Immuno-enzymologique quantitative (E.L.I.S.A.). Lille Médical, 20: 561-566.

Buck, A.A., Sadun, E.H., Anderson, R.I. & Shaffer, E., 1964. Comparative studies of some immunologic screening tests for schistosomiasis in Ethiopia. Amer. J. Hyg., 80: 75-84.

Capel, P.J.A., 1975. The defined antigen substrate spheres (DASS) system and some of its applications. Fifth Int. Conf. on Immunofluorescence and Related Staining Techniques. Ann. N.Y. Acad. Sci., 254: 108-117.

Deelder, A.M., 1973. Immunology of experimental infections with Schistosoma mansoni in the brown mouse and with Fasciola nepolica in the rabbit. Acta Leidensia, 39: 1-107.

Deelder, A.M. & Ploem, J.S., 1974. An immunofluorescence reaction for Schistosoma mansoni using the defined antigen substrate spheres (DASS) system. J. immunol. Methods, 4: 239-251.

Deelder, A.M. & Streefkerk, J.G., 1975. Schistosoma mansoni: Immuno-histoperoxidase procedure in defined antigen substrate spheres (DASS) system as serologic field test. Exp. Parasitol., 37: 405-410.

Ferreira, A.W., Camargo, M.E. & Nakahara, O.S., 1974. Pesquisa de anticorpos anti-Schistosoma mansoni pela técnica anti-globulínica de immunoperoxidase. Rev. Inst. Med. Trop. São Paulo, 16: 341-345.

Ferreira, A.W., Camargo, M.E. & Nakahara, O.S., 1975. Trypanosoma cruzi: Immunoperoxidase antibody test for serologic diagnosis. Exp. Parasitol., 37: 131-137.

Proc. Int. Conf. Schisto. (1975)

Huldt, G., Lagerquist, B., Phillips, T., Draper, C.C. & Voller, A., 1975. Detection of antibodies in schistosomiasis by enzyme-linked immunosorbent assay (ELISA). WHO/SCHISTO/75.38: 1-8.

Kagan, I.G., 1974. Advances in the immunodiagnosis of parasitic infections. Z. Parasitenk., 45: 163-195.

Lichtenberg, F. von, Bawden, M.P. & Shealey, S.H., 1974. Origin of circulating antigen from the schistosome gut: An immunofluorescent study. Amer. J. trop. Med. Hyg., 23: 6, 1088-1091.

Ljungström, I., Engvall, E. & Ruitenberg, E.I., 1974. ELISA, enzyme linked immunosorbent assay — a new technique for sero-diagnosis of trichinosis. Parasitology, Proc. Brit. Soc. for Parasitol., 69: 24.

Madwar, M.A. & Voller, A., 1975. Circulating soluble antigens and antibody in schistosomiasis. Brit. med. J., 1: 435-436.

McCarten, W.G. & Nzelibe, F.K., 1975. Schistosomiasis: Evaluation of the indirect fluorescent antibody, complement fixation, and slide flocculation tests in screening for schistosomiasis. Exp. Parasitol., 37: 239-250.

Moriearty, P.L. & Lewert, R.H., 1974. Delayed hypersensitivity in Ugandan schistosomiasis I. II. III. Amer. J. trop. Med. Hyg., 23: 169-196.

Nash, T.E., 1974. Localization of the circulating antigen within the gut of Schistosoma mansoni. Amer. J. trop. Med. Hyg., 23: 1085-1087.

Nash, T.E., Prescott, B. & Neva, F.A., 1974. The characteristics of a circulating antigen in schistosomiasis. J. Immunol., 112: 1500-1506.

Okabe, K. & Tanaka, T., 1958. A new urine precipitin reaction for schistosomiasis japonica, a preliminary report. Kurume med. J., 5: 45-52.

Oppenheim, J.J., Leventhal, B.G. & Hersh, E.M., 1968. The transformation of column-purified lymphocytes with nonspecific and specific antigenic stimuli. J. Immunol., 101: 262-270.

Ourth, D.D., Matre, R., Helgeland, S.M. & Tönder, O., 1974. An indirect immunoperoxidase test for human antibodies to Toxoplasma gondii. Acta Path. Microbiol. Scand., Section B, 82: 145-150.

Ruitenberg, E.J., Steerenberg, P.A., Boris, B.J.M. & Buys, J., 1974. Serodiagnosis of Trichinella spiralis infections in pigs by enzyme-linked immunosorbent assays. Bull. Wld Hlth Org., 51: 108-109.

Ruitenberg, E.J., Ljungström, I., Steerenberg, P.A. & Buys, J., 1975a. Application of immunofluorescence and immunoenzyme methods in the serodiagnosis of Trichinella spiralis infection. Ann. N.Y. Acad. Sci., 254: 296-303.

Ruitenberg, E.J., Steerenberg, P.A. & Brosi, B.J.M., 1975b. Micro-system for the application of ELISA (enzyme linked immunosorbent assay) in the serodiagnosis of Trichinella spiralis infections. Medikon/Nederland, 4: 30-31.

Sadun, E.H. & Gore, R.W., 1967. Relative sensitivity and specificity of soluble antigens (metabolic and somatic) and whole cercariae in fluorescent antibody tests for schistosomiasis in humans and rabbits. Exp. Parasitol., 20: 131-137.

Sadun, E.H., Williams, J.C. & Anderson, R.I., 1960. Fluorescent antibody technique for serodiagnosis of schistosomiasis in humans. Proc. Soc. exp. Biol., 105: 239-291.

Saver, M.C.V. & Senft, A.W., 1971. Schistosome proteolytic enzyme. Comp. Biochem. Physiol., 38B: 663-678.

Scapin, M. & Tendler, M., 1975. Immunoelectroosmophoresis as a rapid method for detecting soluble antigenic fraction of Schistosoma mansoni. J. Parasitol., 63: 561-562.

Seitz, H.M., 1975. Counter-current immunoelectrophoresis for the demonstration of malarial antigens and antibodies in the sera of rats and mice. Trans. roy. Soc. trop. Med. Hyg., 69: 1, 88-90.

Senft, A.W. & Maddison, S.E., 1975. Hypersensitivity to parasite proteolytic enzyme in schistosomiasis. Amer. J. trop. Med. Hyg., 24: 83-89.

Toussaint, A.J. & Anderson, R.I., 1965. Soluble antigen fluorescent antibody technique. Appl. Microbiol., 13: 552-558.

Voller, A., Huldt, G., Thors, C. & Engvall, E., 1975. New serological test for malaria antibodies. British med. J., 659-661 (March).

Warren, K.S., Kellermeyer, R.W., Jordan, P., Littell, A.S., Cook, J.A. & Kagan, I.G., 1973. Immunologic diagnosis of schistosomiasis. I. A controlled study of intradermal (immediate and delayed) and serologic tests in St. Lucians infected with Schistosoma mansoni and in uninfected St. Vincentians. Amer. J. trop. Med. Hyg., 22: 189-198.

Williams, J.S., Sadun, E.H. & Gore, R.W., 1971. A radioactive antigen microprecipitin (RAMP) assay for schistosomiasis. J. Parasitol., 57: 2, 220-232.

Wilson, J., Sulzer, A.J. & Walls, K.W., 1974. Modified antigens in the indirect immunofluorescence test for schistosomiasis. Amer. J. trop. Med. Hyg., 23: 1072-1076.

Wolfson, R.L., Maddison, S.E. & Kagan, I.G., 1972. Migration inhibition of peripheral leukocytes in human schistosomiasis. J. Immunol., 109: 123-128.

SUBCOMMITTEE SESSIONS

IMMUNE MECHANISMS IN EXPERIMENTAL MURINE SCHISTOSOMIASIS

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The development of protective immunity in man following infection with one or more species of *Schistosoma* is suggested by epidemiological evidence and by the results of a few small uncontrolled studies. In experimental animal models the state of concomitant immunity which develops is dependent on the presence of adult worms (Smithers, 1962). Attempts to vaccinate animals with extracts of larval or adult stages of the parasite have essentially been unsuccessful.

The studies of Colley (1972), Green & Colley (1974) and Sher et al. (1975) have contributed to the demonstration, in the mouse, by Mahmoud et al. (1975a, 1975b), of the eosinophil as the effector cell in the specific antibody-mediated cytotoxicity, which is directed at the schistosomules very early after challenge of an immune mouse.

In the rat, Phillips et al. (1975) have shown the protective mechanisms to be more complex, with modulation of cellular and humoral immunity during the course of infection. Numerous studies in the monkey model have shown that the passive transfer of serum alone will not confer protection (Maddison et al., 1976 ; Smithers, 1976). We demonstrated a significant reduction in clinical disease and in worm burdens of Rhesus monkeys which received a combination of hyperimmune serum and transfer factor (Maddison et al., 1976).

We now present the findings of our preliminary studies with a *Schistosoma mansoni* mouse model. We investigated the effect of transfer of cells and/or serum from mouse donors infected for varying periods of time on the worm burdens of the recipients following challenge.

Materials and Methods

Spleen and lymph node cells were obtained from CBA mice infected for 4-15 weeks after exposure to 50-80 cercariae. Cells from uninfected donors were used in some of the controls.

Hyperimmune serum was prepared by infecting mice with 50 cercariae. Ten to 12 weeks later the mice were reexposed to an additional 50 cercariae. The animals were exsanguinated 18-22 weeks after the initial infection. Serum from uninfected mice was used in some of the controls.

Each mouse received 2-3 $\times$ 10^8 small mononuclear spleen cells or 0.7-3 $\times$ 10^8 small mononuclear mesenteric lymph node cells by intraperitoneal injection. In most experiments the cells were given 3 weeks before challenge but in the last two groups of experiments when cells were given 2 days before challenge, they were also efficacious.

Serum recipients received 1 ml by intraperitoneal injection on day —1 rela-

tive to challenge with cercariae and a similar dose on day +5. In one group of experiments, however, a single dose of serum given on day —1 was effective.

The numbers of mice in each group varied from 6 to 16. Recipients were exposed to cercariae by subcutaneous injection (Peters & Warren, 1969). Worm burdens were determined 7 weeks after infection.

In our model previous infection for 10 weeks conferred partial pr tection to a challenge exposure of 100 cercariae as evidenced by an 83% reduction in worm burden compared with previously uninfected controls (Table 1). The passive transfer of immune serum alone did not result in a significant reduction of the worm burdens in three groups of mice (Table 1).

Transfer of normal cells alone did not confer protection in any of four experiments. Normal spleen cells combined with immune serum also failed to protect (Table 2).

Transfer of spleen cells from donors infected for 10 weeks plus transfer of

normal serum resulted in partial protection of the recipients in one of two experiments. This equivocal result requires further study. In four experiments in which cells from donors infected for periods varying from 6 to 15 weeks were transferred without serum, no increased resistance to challenge infection was observed (Table 3).

Cells from donors infected from 4 to 10 weeks in combination with hyperimmune serum caused a significant reduction of worm burdens in eight experiments (Table 4). When cells were collected from donors infected for 15 weeks, however, they were apparently no longer effective in transferring the cellular component of functional immunity to recipients. This apparent loss of competence may not reflect a change in the immunologic status of the activated cells, but may signify development of an inhibitor at this stage of infection in the mouse. Such an inhibitor could conceivably be a subpopulation of suppressor lymphocytes. Again, this facet of our findings requires further study.

TABLE 1. Effect of previous infection or transfer of hyperimmune serum of challenge infection with *Schistosoma mansoni*

Treatment of recipients	No. of mice	No. of cercariae	Mean worm burden + SEM	% Reduction worms	P
Nil	8	100	34.0 ± 4.2		
Previous exposure	7		5.7 ± 1.5	83	<0.01
Nil	6		31.7 + 4.6		
Immune serum	9		24.9 ± 3.8	21	NS
Immune serum	8	100	30.8 ± 4.0	3	NS
Immune serum	7		22.9 ± 4.3	28	NS

SEM = Standard error of mean.

NS = Not significant.

TABLE 2. **Effect of adoptive transfer of normal cells, with and without serum, on challenge infection with** *Schistosoma mansoni*

Cells	Pretreatment Serum	No. of mice	No. of cercariae	Mean worm burden+SEM	% Reduction worms	P
Nil	Nil	9	100	24.6 ± 2.5		
SP	Nil	8		15.8 ± 2.7	36	NS
Nil	Nil	12		12.2 ± 1.5		
SP	Nil	10		13.5 ± 1.7	0	NS
SP	Immune	10	50	8.0 ± 1.7	34	NS
LN	Nil	9		14.5 ± 1.8	0	NS
Nil	Nil	14		17.9 ± 2.1		
SP	Nil	9	100	21.6 ± 2.7	0	NS
SP	Immune	8		15.4 ± 2.8	14	NS
LN	Nil	10		12.2 ± 2.5	32	NS
Nil	Nil	13	60	29.2 ± 1.9		
SP	Nil	7		27.4 ± 2.6	6	NS

SP = Spleen cells   SEM = Standard error of mean

LN = Mesenteric lymph node cells   NS = Not Significant

TABLE 3. **Effect of adoptive transfer of sensitized cells only or with normal serum on challenge infection with** *Schistosoma mansoni*

Cells	Pretreatment Serum	No. of mice	No. of cercariae	Mean worm burden+SEM	% Reduction worms	P
Nil	Nil	12	50	12.2 ± 1.5		
SP 6 weeks	Nil	11		13.7 ± 1.6	0	NS
Nil	Nil	14	100	40.1 ± 2.7		
LN 8 weeks	Nil	8		30.5 ± 3.6	24	NS
Nil	Nil	7	100	39.1 ± 3.3		
SP 10 weeks	Normal	17		26.0 ± 2.1	34	<0.01
Nil	Nil	16	100	18.2 ± 2.1		
SP 10 weeks	Normal	9		22.4 ± 2.6	0	NS
Nil	Nil	9	100	24.6 ± 2.5		
SP 10 weeks	Nil	9		18.2 ± 2.6	26	NS
LN 10 weeks	Nil	7		20.3 ± 2.9	17	NS
Nil	Nil	7	100	16.7 ± 3.5		
SP 15 weeks	Nil	9		20.0 ± 3.1	0	NS

SP = Spleen cells   SEM = Standard error of mean

LN = Mesenteric lymph node cells   NS = Not Significant

TABLE 4. Effect of adoptive transfer of sensitized cells and immune serum on challenge infection with *Schistosoma mansoni*

Pretreatment Cells	Serum	No. of mice	No. of cercariae	Mean worm burden+SEM	% Reduction worms	P
Nil	Nil	11	50	19.5 ± 2.1		
SP 4 weeks	Immune	10		9.6 ± 2.2	51	<0.01
Nil	Nil	12	50	12.2 ± 1.5		
SP 6 weeks	Immune	10		2.4 ± 1.7	80	<0.01
Nil	Nil	14	100	40.1 ± 2.7		
SP 8 weeks	Immune	10		17.4 ± 3.3	57	<0.01
Nil	Nil	7	100	39.1 ± 3.9		
SP 10 weeks	Immune	10		22.5 ± 3.4	43	<0.01
LN 10 weeks	Immune	10		25.7 ± 2.1	34	<0.01
Nil	Nil	9	100	24.6 ± 2.5		
SP 10 weeks	Immune	8		13.3 ± 2.7	46	<0.01
Nil	Nil	16	50	18.2 ± 2.0		
SP 10 weeks	Immune	11		10.7 ± 2.4	41	<0.05
LN 10 weeks	Immune	8		12.2 ± 2.8	33	NS
Nil	Nil	9	60	27.4 ± 2.0		
SP 10 weeks	Immune	10		18.4 ± 1.9	33	<0.05
LN 10 weeks	Immune	7		5.7 ± 2.3	79	<0.01
Nil	Nil	13	60	29.2 ± 1.9		
SP 10 weeks	Immune	10		17.4 ± 2.2	40	<0.01
LN 10 weeks	Immune	7		16.4 ± 2.6	44	<0.01
Nil	Nil	7	100	16.7 ± 3.5		
SP 15 weeks	Immune	9		18.6 ± 3.1	0	NS
LN 15 weeks	Immune	7		16.6 ± 3.5	0	NS
Nil	Nil	6	75	18.2 ± 5.9		
SP 15 weeks	Immune	9		21.8 ± 4.8	0	NS
LN 15 weeks	Immune	11		16.5 ± 4.4	9	NS

SP = Spleen cells

LN = Mesenteric lymph node cells

The requirement of activated cells in addition to hyperimmune serum for the transfer of protective immunity in the mouse differs from the observations of Sher et al. (1975), which were subsequently confirmed by Mahmoud et al. (1975b), that hyperimmune serum alone will confer protection. However, our model differs in two ways from the model of Sher et al. (1975). The latter gave serum intravenously and challenged by percutaneous penetration of the cercariae. We gave serum intraperitoneally and challenged by subcutaneous injection of cercariae. Later histologic studies by von Lichtenberg et al. (1976) indicate that the schistosomules of the challenge infection are probably rapidly killed (within 1-3

days) in the skin by the mechanism of antibody-mediated cytotoxicity with eosinophils as the effector cells. Our model bypasses this defence barrier which occurs in the skin. The protection which we observed may possibly be more complex, occurring at a later stage in the development of the challenge infection in the mouse host. Experiments to investigate this hypothesis have been initiated.

In attempts to characterize the immunologic competence of our donors, we carried out lymphocyte transformation and footpad skin test studies. Our preliminary results, shown in Table 5, suggest that the ability of mouse donor lymphocytes to transform in the presence of the *S. mansoni* antigen used does not correlate with their ability to participate in

protective immunity. Cells from donors infected for 6 weeks when combined with hyperimmune serum conferred protection but did not show significant *in vitro* transformation. Conversely, cells from old infections did transform, but we were unable to protect with cells from donors infected for 15 weeks.

Similarly, footpad skin tests did not show correlation with skin reactivity of immediate, Arthus, or delayed types with the ability of either cells or serum from the respective donors to contribute to protective immunity. Footpad reactivity was assessed either by measuring the amount of inflammatory response as indicated by the extravasation of ^{125}I-BSA* 1 hour after intravenous injection of the isotope or by measurement of footpad thickness.

TABLE 5. Lymphocyte transformation of cells from uninfected controls and mice infected for varying periods with *Schistosoma mansoni*

Duration of infection	No. of cercariae	Stimulation ratio S. mansoni 50μ	PPD 20μ
Uninfected		1.9	3.6
Uninfected		1.1	ND
Infected 6 weeks	80	1.3	ND
Uninfected		1.4	3.1
Infected 8 weeks	75	7.3	2.1
Uninfected		0.6	ND
Infected 9 weeks	80	10.8	ND
Uninfected		3.2	4.2
Exposed X2 21 weeks	50 + 60	26.0	3.2
Uninfected		1.5	ND
Exposed X2 23 weeks	50 + 60	17.3	ND

PPD = Purified protein derivative of *Mycobacterium tuberculosis*

ND = Not done

* Radioactively labelled bovine serum albumin.

The results of footpad studies carried out on mice infected for 4 weeks are shown in Table 6. The ratio of isotopic measurements was 1.7 at half an hour after antigen injection and only 1.1 at 24 hr. To characterize the results of the radioisotope footpad assay, we examined other groups of similarly infected mice for increase in footpad thickness and, histologically, for cellular infiltration in the footpads. In addition, serological responses indicative of immediate and Arthus skin reactivities, that is, passive cutaneous anaphylaxis (PCA) and complement fixation (CF), respectively, were also tested. After infection for 4 weeks, injection of antigen resulted only in a slight increase in footpad measurements after half an hour. However, histological studies showed characteristic reactions of immediate, Arthus and delayed hypersensitivities. The sera of the majority of animals tested were positive in the PCA test, but the complement fixation test was less sensitive than the *in vivo* skin test and histological examination.

TABLE 6. Footpad skin tests results on mice infected for 4 weeks after exposure to 85 cercariae of *Schistosoma mansoni*

Hours after antigen injection	Ratio ^{125} I-BSA in antigen and control feet	Difference in measurement between antigen and control feet	Histology
0.5	4*/1.7**	30*/0.69***	Immediate
6	4/1.7	18/0.32	Arthus
12	4/1.4	18/0.22	Residual Arthus
24	4/1.1	12/0.27	Weak-delayed
48	4/0.9	6/0.32	Weak-delayed

* = No. of mice	PCA : 7/10 positive	
** = Ratio	CF : 1/8 positive	
*** = Millimeters		

Table 7 shows the results of similar studies on mice infected for 12 weeks. The radioisotope assay showed inflammatory responses at 30 min and 6 hr but did not detect the delayed response. Again, footpad measurements were unsatisfactory for detecting the hypersensitivity responses.

These transformation and skin test results reflect responses to our adult worm antigen. Although adult worms have been shown to be part of the protective stimulus in the monkey, the antigenic components present in our experiments may not be involved in any of the immunologic mechanisms of protective

TABLE 7. Footpadskin tests on control mice and mice infected for 12 weeks after exposure to 45 cercariae of *Schistosoma mansoni*

Status of mice	Hours after antigen injection	Ratio 125 I-BSA in antigen and control feet	Difference in measurement between antigen and control feet Histology	
Control	0.5	ND	9*/0.13***	Nil
Infected		4*/2.0**	8/0.75	Immediate
Control	4	ND	9/0.06	Nil
Infected		4 /1.3	9/0.53	Early Arthus
Control	6	2 /1.2	9/0.04	Nil
Infected		2 /2.5	9/0.31	Arthus
Control	12	2 /0.9	2/0.23	Nil
Infected		2 /0.9	6/0.42	Residual Arthus
Control	24	2 /0.9	2/0.23	Nil
Infected		2 /1.2	2/0.25	Delayed

* = No. of mice
** = Ratio
*** = Millimeters
ND = Not done

Controls : PCA and CF negative

Infected : PCA and CF positive

immunity. This hypothesis is supported by the numerous reports of failure to confer protection by injection of extracts of adult worms.

In conclusion, our findings suggest that the protective immune mechanisms in the murine model of schistosomiasis may be complex. Identification of the functional antigens would facilitate the understanding of the mechanisms involved.

LITERATURE CITED

Colley, D.G., 1972. Schistosoma mansoni: Eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. Exp. Parasit., 32: 520.

Green, B.M. & Colley, D.G., 1974. Eosinophils and immune mechanisms. II. Partial characterization of the lymphokine eosinophil stimulation promoter. J. Immunol., 113: 910.

Maddison, S.E., Hicklin, M.D. & Kagan, I.G., 1976. Schistosoma mansoni: Reduction in clinical manifestations and in worm burdens transferred by serum and transfer factor from immune and normal rhesus monkeys. Exp. Parasit., 39: 29.

Mahmoud, A.A.F., Warren, K.S. & Graham, R.C., 1975a. Antieosinophil serum and the kinetics of eosinophilia in Schistosomiasis mansoni. J. exp. Med., 142: 560.

Mahmoud, A.A.F., Warren, K.S. & Peters, P.A., 1975b. A role for the eosinophil in acquired resistance to Schistosoma mansoni infection as determined by antieosinophil serum. J. exp. Med., 142: 805.

Peters, P.A. & Warren, K.S., 1969. A rapid method of infecting mice and other laboratory animals with Schistosoma mansoni: Subcutaneous injection. J. Parasit., 55: 558.

Phillips, S.M., Reid, V.A., Bruce, J.I., Hedlund, K., Colvin, R.C., Cambell, R., Diggs, C.L. & Sadun, E.H., 1975. The cellular and humoral immune response to Schistosoma mansoni injection in inbred rats. I. Mechanisms during initial exposure. Cell. Immunol., 19: 99.

Sher, A., Smithers, S.R. & Mackenzie, P., 1975. Passive transfer of required resistance to Schistosoma mansoni in laboratory mice. Parasit., 70: 347.

Smithers, S.R., 1962. Stimulation of acquired resistance to Schistosoma mansoni in monkeys: role of eggs and worms. Exp. Parasit., 12: 263.

Smithers, S.R., 1976. Immunity to trematode infections with special reference to schistosomiasis and fascioliasis. In: Cohen, S. & Sadun, E.H. (Eds.), «Immunology of Parasitic Infections». Blackwell's Scientific Publications, Oxford and London. 297 p.

von Lichtenberg, F., Sher, A., Gibbons, N. & Doughty, B.L., 1976. Eosinophil enriched inflammatory response to schistosomula in the skin of mice immune to Schistosoma mansoni. Amer. J. Path., 84: 479.

FURTHER DISCUSSION ON THE NEW HYPOTHESIS FOR THE MECHANISM OF IMMUNITY TO SCHISTOSOME INFECTION*

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While the existence of immunity to schistosome infection was noticed more than 50 years ago, the mechanism of immunity to this infection remains obscure (Hsü et al., 1975). A full understanding of the mechanism of immunity to schistosome infection is important, however, not only in theory but also in practice, viz., in immunoprophylaxis, immunotherapy, etc. Although the complementary value of immunoprophylaxis (schistosome vaccination) to snail eradication in the control of schistosomiasis has recently been recognized (Hsü & Hsü, 1974), the development of vaccine against schistosome infection is, unfortunately, still only in the investigative stages. The major difficulty is that we have no knowledge of the type of immune response which is mainly involved in initiating and effecting the resistance against schistosome infection. If we can obtain such knowledge, we may direct our study of schistosome vaccine ,live or killed, towards the development of this type of response. Our newly published hypothesis for the mechanism of immunity to schistosome infection (Hsü & Hsü, 1975a) was made to accelerate the understanding of the type of immunity involved in the resistance to this infection. The new hypothesis is as follows :

«The mechanism of immunity seems to be initiated by cell-mediated immunity (CMI) with possible subsequent cooperation of humoral antibodies. The immunologically committed T lymphocytes occurring in the CMI may perform the following functions : 1) they produce lymphokines to inhibit the migration of macrophages and to activate them to form epithelioid cells and giant cells which will destroy schistosomula in due time ; 2) they produce eosinophil chemotactic factor to attract eosinophils which will destroy schistosomula faster than the macrophages and giant cells ; and 3) they perform the helper function to enable the B cells to produce antibody which, in turn, either manifests a cytotoxic effect on the schistosomula, or opsonizes the migrating schistosomula in the epidermis and dermis to be destroyed by eosinophils, or directly activates the eosinophils to destroy the schistosomula».

The above hypothesis is based mainly upon histopathological studies of the skin lesions of rhesus monkeys which were immunized with cercariae of *Schistosoma japonicum* exposed to high doses of X-irradiation and also of the skin lesions of the immunized monkeys challenged with normal cercariae (Hsü et al., 1975). The

resistance produced by this method of immunization has been demonstrated to be strong (Hsü et al., 1969, 1975). Although there is a limitation in interpreting purely histological findings in terms of complicated functional interactions between cells in the mechanism of immunity to schistosome infection, this investigation may serve as a working hypothesis for further experimental studies. For the details of the results of the study upon which the hypothesis is based and for which the present discussion is made, our previous publication (Hsü et al., 1975) should be consulted. The essential points of the hypothesis to be discussed in this paper are as follows :

CMI as Initiator of the Immune Mechanism

As previously described (Hsü et al., 1975), delayed hypersensitivity (DH) in the immunized monkeys was first noticed at Day 7 of the first immunization. Macroscopically, the lesion appeared as a defined erythematous induration in the skin. Microscopically, there was typical perivascular infiltration of mononuclear cells in the dermis. In the additional immunizations, the lesions were noticed at the end of Day 1 and the climax of the lesions was also accelerated by the number of immunizations. With the subsequent development of epithelioid cells and multinucleated giant cells, granuloma formation appeared, and the schistosomula were observed to be destroyed by these cells. These findings indicate that in this process of immunization, the mechanism of immunity was initiated by DH or CMI. As is going to be discussed below, other important immunological processes develop sequentially, but all of them are connected or may be connected with the CMI, the initiator of this immune mechanism.

It is of interest to note that while the presence of DH in human schistosomiasis was reported more than 40 years ago (Khalil & Hassan, 1932), a meaningful study of the relation of this reaction to the mechanism of immunity has been renewed only recently (Pellegrino & Memoria, 1960 ; Sadun & Biocca, 1962 ; Wolfson et al., 1969, 1972 ; Maddison et al., 1970, 1972, 1973 ; Colley et al., 1972 ; Vernes et al., 1972a, b, c, 1973 ; Capron et al., 1973 ; Warren et al. 1973 ; Moriearty & Lewert, 1972a, b, c ; WHO, 1973 ; Hsü et al., 1974). In their study on DH in schistosomiasis, Moriearty & Lewert (1974 b) reported that their findings were consistent with the hypothesis that DH frequencies in a group from an endemic area are related to the frequency of p·r· exposure to schistosome infection in the group, and Wolfson et al. (1969) speculated that the state of DH confers greater immunity to infection than the presence of circulating antibodies. In their study of the lethal factor of sera from schistosomiasis patients on schistosomula and of the results of hypersensitivity tests, Capron et al. (1973) noticed a quite unexpected relationship between the lethal factor and DH. In a recent WHO report prepared by an international group of experts on CMI and resistance to infection (WHO, 1973), the statement was made that in schistosomiasis, resistance is cell-mediated, because little correlation has been observed in man between antibody levels and the apparent degree of resistance. Drawing mainly upon Colley's work on the cell-mediated hypersensitivity (CMH) induced by the soluble antigen of schistosome eggs (Colley, 1971, 1972a, b ; Buchanan et al., 1973 ; Fine et al., 1973), Larsh & Weatherly (1975) tried to show the importance of CMI in schistosomiasis in their review of the role of CMI against certain parasitic worms. However, CMH does not always result in CMI (Crowle,

1972) while CMI is protective, CMH can be either protective or destructive. Warren (1972) was right to say that the granulomatous CMH elicited by schistosome eggs plays no role in the development of CMI (Warren, 1972) ; this statement is well supported by the fact that the eggs have not been demonstrated to play a role in schistosome immunity (Smithers & Terry, 1969). In fact, this granulomatous CMH is not only nonprotective, it is destructive because lesions resulting from this reaction will produce detrimental hepatosplenic schistosomiasis (Larsh & Weatherly, 1975). On the other hand, these authors made a very fine proposal regarding the role of CMI in schistosome immunity although the way by which the DH or the CMI is involved in the mechanism of immunity to schistosomiasis was not elucidated. This is evidently a difficult problem. When Moriearty & Lewert (1974a) reported their work on DH in Ugandan schistosomiasis, they hoped that the skin test for DH could be used in studying the resistant state of the human host, but in the last article of their series (1974c), they said that it was not possible in their investigation to assess the relationship between DH and an immune or resistant state in the host. Accordingly, the present finding of the development of the CMI in immunized monkeys may be regarded as the first cogent evidence showing the manner and the extent to which the CMI is involved in the mechanism of immunity to schistosomiasis. However, as the present results are based only on histopathological examinations of the immunized tissues, further experimental work to establish the function of the T cells in this case should be carried out.

There is one important point regarding the role of CMI in schistosomiasis which especially needs discussion. If immunity to schistosome infection is indeed cellular, it should be transferable by cells. Two reported attempts to transfer immunity by cells were without success (Hunter et al., 1967 ; Maddison et al., 1970). If, however, one realizes that the mechanism of immunity to schistosomiasis, as postulated in the hypothesis and discussed further below, is not a simple cellular immunity, one may understand why a simple transfer of common lymphocytes failed to produce protective immunity. We believe that the adoptive transfer of cellular immunity to schistosomiasis eventually will be successful, if the method of cell transfer is improved by additional considerations of the associated factors. (We are glad to insert here the personal information received from Dr. S.M. Phillips that he and his co-workers (Phillips et al., 1975) have recently succeeded in obtaining a significant immune effect against the challenge infection of *S. mansoni* in the rat by adoptive transfer of thymus-dependent lymphocytes from immunized hosts. The protection was observed when the transferred cells were obtained from animals exposed to cercariae 3-4 weeks earlier. This timing in obtaining the cells from the immunized donor seems to be a special point in their findings).

In recent years, CMI or DH has been recognized to be important in the mechanism of immunity in a number of helminthic infections, viz., *Trichostrongylus colubriformis* (Waagland & Dineen, 1965), *Nippostrongylus brasiliensis* (Keller & Keist, 1972), *Trichinella spiralis* (Larsh, 1967), *Litomosoides carinii* (Bagai & Subrahmanyam, 1970), *Ancylostoma caninum* (Miller, 1967), *Fasciola hepatica* (Long, 1967), and *Hymenolepis nana* (Okamoto & Kiozumi, 1972). Although CMI was reported to play a role secondary to antibody mediated immunity in the mechan-

ism of immunity to *Nippostrongylus brasiliensis* (Keller & Keist, 1972), this factor seems to take a main role in, or to act as an initiator of, the immune mechanism in the other helminthic infections. In the case of *Trichostrongylus colubriformis* infection, there was, secondary to lymphocytes, a sequential involvement of other cellular factors such as eosinophils and basophils (Rothwell & Dineen, 1972). This indicates that CMI is indeed important in the mechanism of immunity to helminthic infection. It further shows that the reactions involved are oftentimes complicated during the course of the development of the immunity and diversified in manifestations in different helminthic species.

The Role of Eosinophils in the Immune Mechanism

The results of histopathological studies of the dermal tissues of our rhesus monkeys immunized with cercariae of *Schistosoma japonicum* exposed to high doses of X-irradiation (Hsü et al., 1975) were also interesting with regard to eosinophils. In the first immunization, eosinophils were seen only occasionally. However, eosinophils became very prominent at Day 7 in the second immunization. At first appearance, they were well intermixed with the mononuclear cells in the perivascular infiltration or around the entrapped schistosomula in the dermis, indicating a possible chemotactic effect of the mononuclear cells. Interestingly, the time of appearance and the peak intensity of the eosinophils were accelerated by the number of immunizations. In their studies of eosinophils in rats infected with *Trichinella spiralis*, Basten and his associates (Basten & Beeson, 1970 ; Basten et al., 1970) reported that the eosinophil response belongs in the category of immunological reactions and that lymphocytes play a role in the in-

duction of this response. In mice infected with *S. mansoni,* Colley (1972) and Fine et al. 1973) reported a pronounced eosinophilia at the time of peak lymphoid blastogenic responsiveness induced by newly deposited eggs. They considered their finding to be further evidence for the importance of T-lymphocytes in the evocation of blood eosinophilia as originally demonstrated in experimental trichinosis. The present finding of the accelerated appearance of eosinophils in successive immunizations and the intimate association of eosinophils and mononuclear cells in the schistosome lesions is an additional support for Basten's view. The interesting relationship between DH and eosinophils in the skin was first discovered by Arnason & Waksman (1963) and later it was enthusiastically worked out by Cohen (Cohen & Ward, 1971 ; Cohen et al., 1974 ; Leber et al., 1973 ; Torisu et al., 1973). The present study is another illustration of such a close relationship. It may also be appropriate to mention that in the challenge infection of *Trichostrongylus colubriformis* in previously immunized guinea pigs, eosinophils developed in the parasitic sites as a consequence of the DH reaction (Rothwell & Dineen, 1972). The prevalence of eosinophils in the wake of DH may not be a rare occurrence in the mechanism of immunity to helminthic infections.

It has been demonstrated that an eosinophil chemotactic factor (ECF) may be generated by the interaction of a soluble factor derived from antigen-activated lymphocytes (ECF_p) with performed antigen-antibody complexes (Cohen & Ward, 1971). However, Colley (1973) reported that following *in vitro* exposure of sensitized lymphoid cells (obtained from lymph nodes of mice infected with *S. mansoni*) to a soluble immunogenic schistosomal egg preparation, the super-

natant culture medium induces increased migration of eosinophils, and the activity-producing substance was regarded as a lymphokine which was designated eosinophil stimulation promoter (ESP). Although both ECF and ESP are lymphokines, ECF involves a further reaction with the antigen-antibody complexes than does ESP. It will be of interest to find out whether in the present case, the production of the eosinophil chemotactic factor from the interaction of the sensitized lymphocytes and the schistosomular antigen requires an additional action of schistosomular antigen-antibody complexes. In addition to the ESP (or ECF) which originates from the sensitized thymus-dependent lymphocytes, there is strong circumstantial evidence from the results of the study presently under consideration to indicate the presence of another eosinophil chemotactic factor, i.e. the eosinophil chemotactic factor of anaphylaxis (ECF-A). When the well-immunized monkey was challenged, the reaginic whealing reaction and Arthus-like reaction appeared before the onset of the DH reaction, i.e. before the appearance of the perivascular infiltration of mononuclear cells. In both the reaginic whealing reaction and the Arthus-like reaction, the important reacting cells were eosinophils. In these cases, it is difficult to assume that the eosinophils were evoked by mononuclear cells. It has been reported that the ECF-A was released from mast cells by IgE antigen-antibody reaction (Kaliner et al., 1973 ; Wasserman et al., 1974). Because the degranulation of mast cells was commonly seen in the dermis at 15 min after the challenge of the well-immunized monkey (Hsü et al., 1974), and because the IgE antibody was demonstrated in the dermal tissue of this animal (Hsü & Hsü, unpubl.), the eosinophils in the epidermis and dermis that appeared in the first day of challenge

may be assumed to be the result of the action of the ECF-A. Thus, in the well-immunized monkey, the eosinophil in the dermal tissues may be induced at different periods by two kinds of chemotactic factors, the ECF-A and ESP (or ECF). These results also show that humoral antibodies were gradually becoming involved in the mechanism of immunity to schistosomiasis.

With progressive immunizations, there was a shortened survival of schistosomula in the skin. The migrating schistosomula seemed to be attacked by cells of the mononuclear cell series and eosinophils. As mentioned above, cells of the mononuclear cell series seemed to play the prominent role in the first immunization. With subsequent immunizations there was a progressively greater contribution of eosinophils to the reaction, along with a diminution in the number of participating mononuclear cells and giant cells. With repeated immunizations and following challenge, this transition occurred progressively earlier in the course of the reaction and was associated with accelerated schistosomular destruction. As no giant cells were encountered in the challenged monkey, eosinophils most probably had taken over the role of destroying the migrating schistosomula in the skin. Results of our previous studies (Hsü et al., 1971, 1974) also showed that eosinophils play an important role in the schistosomulicidal action. These cells are known to contain a number of lysosomal granules which produce several kinds of hydrolytic enzymes. We may assume that when a schistosomulum is trapped by an infiltration of eosinophils, a great number of these granulocytes will disintegrate and the lysosomal hydrolytic enzymes will be released. Cotran & Litt (1969) observed that in some preparations, a reaction product of

the peroxidase of eosinophils was found extracellularly between the surface membrane of contiguous cells. It will be of importance to investigate the way in which the enzymes from the eosinophils act upon the trapped schistosomula. Mention should be made that in the challenge infection of *Trichostrongylus colubriformis* in guinea pigs, eosinophils which were produced as a consequence of DH were found to produce histamine, enabling the expulsion of the challenge infection (Rothwell et al., 1971). Evidently, in one way or the other, eosinophils play a role in the effector mechanism of the immune response.

Humoral Antibodies and T-B Cell Co-operation

Although humoral antibodies were not studied in our previous works (Hsü et al., 1974, 1975), the formulation of a sound working hypothesis of the mechanism of immunity to schistosome infection requires a brief discussion of the possible roles of the functional antibodies. Antischistosomular antibodies may be involved in four events : (a) in ECF, (b) in ECF-A, (c) as an opsonin, and (d) as cytotoxic antibody. As mentioned above, we are not quite certain whether the lymphokine produced by the thymus-dependent lymphocytes for attracting the eosinophils during the immunization is ECF or ESP. Should it be ECF, the antibody will also be involved in the production of the eosinophil chemotactic factor. On the other hand, IgE antibody is assumed to initiate the reaction which results in the formation of ECF-A. The presence of opsonin in the sera of schistosome-infected hosts has been recorded and the attracted cells found to be neutrophils (Newsome, 1962 ; Dean et al., 1974). Should the opsonin also act on schistosomula in the present cases, the attracted cells would be mainly eosinophils. Re-

cently, the presence of cytotoxic antibody (lethal to schistosomula 1-4 days old) in the sera of infected hosts has been reported (Capron et al., 1973 ; Clegg & Smithers, 1972 ; Dean et al., 1974, 1975 ; Murrell & Clay, 1972). Results of *in vitro* experiments made in our laboratory showed that in the cultivation of schistosomula of *S. japonicum* in the hyper-immune serum from a well-immunized monkey, most of the schistosomula were found dead in one day while only a small percentage of schistosomula cultured in control serum were found dead (Hsü & Hsü, unpubl.). These results coincide with those obtained from our histopathological examinations : after the well-immunized monkey was challenged with normal cercariae, most of the migrating schistosomula, surrounded by eosinophils, disintegrated in the first day. Evidently, in the serum of the well-immunized monkey, there is a strong cytotoxic antibody which may work synergically with the eosinophils to destroy the schistosomula in their early life. However, efforts to transfer passive resistance with immune serum by previous workers have usually failed. Although a partially successful transfer of acquired resistance to *S. mansoni* has recently been accomplished in mice by Sher et al. (1975), it is still questionable to attribute the *in vivo* schistosomulicidal effect to the same reaction of the cytotoxic antibody that has been demonstrated *in vitro*, viz., (1) although cytotoxic antibody against *S. mansoni* may be induced in the serum of rats by injection of adult worm membranes, it does not confer immunity to a challenge infection, (Sher et al., 1974) and (Hsü & Hsü, 1974), the cytotoxic activity against *S. mansoni* induced in rats still remains high in the serum even when the protective immunity has declined and disappeared (Perez et al., 1974). As the effective action of the cytotoxic antibody *in vivo* may involve a num-

ber of associated factors, it is possible that some of these factors may enhance and some may inhibit the schistosomulicidal effect. Some factors may be cooperative or synergistic. In addition, during the course of the immunological development, a kind of enhancing antibody may arise (Phillips et al., 1975). The *in vivo* results will depend upon the balance of these factors. We have to investigate more of these factors before a proper interpretation can be made. Dean et al. (1974, 1975) have studied the combined schistosomulicidal effect of immune serum and leukocytes from rats and guinea pigs infected with *S. mansoni* and reported that the combined effect was stronger than that of the immune serum alone (Dean et al., 1974, 1975). In both host species, the most important reacting leukocytes were the neutrophils. These authors emphasized that with guinea pigs, the addition of eosinophils and macrophages did not increase the level of the killing effect of the antiserum although neutrophils did (Dean et al., 1975). Since results obtained from our histopathological examination (Hsü et al., 1975), as mentioned above, strongly indicated that eosinophils were involved in the schistosomulicidal action, experimental work has recently been performed in our laboratory to investigate the combined effect of immune serum and eosinophils from immunized monkeys on *S. japonicum* schistosomula. Our results (Hsü & Hsü, unpubl.) do show their synergistic schistosomulicidal effect.

In our laboratory (Hsü & Hsü, unpubl.), we have demonstrated the presence of IgG (with the involvement of complement) and IgE in the tissue of the skin of a well-immunized monkey. The above described cytotoxic antibody has been reported to be IgG (Dean et al., 1974, 1975). The function of IgE in the immune mechanism should be investigated further.

The reaginic reaction can reasonably be assumed to be caused by the IgE antibody. The possibility that the Arthus-like reaction observed in our immunized monkey was elicited by the IgE antibody has also been suggested (Dolovich et al., 1973 ; Hsü et al., 1974). Because most schistosomula in the well-immunized monkey were destroyed in the first day (Hsü & Hsü, 1975b), it is important to find out whether, in addition to its above-mentioned function in inducing the ECF-A reaction, the IgE also plays a role as a cytotoxic antibody.

The above-described events indicate that the mechanism of schistosome immunity is a very complicated one. It may involve a sequence of several systems of immunological reactions, interactions and cooperation. The T cells initiate the mechanism of immunity to schistosome infection. Therefore, it is possible that they act to regulate the immune mechanism in such a way that they render a helper function to the B cells in producing the functional schistosomulicidal antibodies. In this context it is of great interest to mention that in the passive cutaneous anaphylaxis (PCA) reaction of mice infected with *S. mansoni*, Fine et al. (1973) demonstrated that the formation of reaginic antibody to the egg soluble antigen was made by the presence of T-lymphocytes, offering evidence of T-B cell interaction. It is hoped that further experimental investigations will prove the validity of this portion of the hypothesis.

Conclusion

If this new hypothesis that the CMI is the type of immune response which is involved in initiating the mechanism of immunity to schistosome infection can be verified, then future research on the development of vaccine against schistosomiasis should be aimed towards inducing

the CMI. In diseases caused by micro-organisms, delayed hypersensitivity or cell mediated immunity is not generally developed by killed vaccine but by infection with avirulent organisms or by recovery from the disease, i.e. by the sojourn of living organisms in vaccinated hosts for a certain time. However, the induction of CMI by killed organisms is not entirely impossible: in tuberculosis the dead tubercle bacilli are active both as immunizing and sensitizing agents. It is obvious that in developing a potent schistosomiasis vaccine, either live or killed, it is important that the vaccine should be able to induce the T cells to initiate the CMI and be followed by T-B cell cooperation to complete the immune mechanism.

LITERATURE CITED

Arnason, B.G. & Waksman, B.H., 1963. The retest reaction in delayed sensitivity. Lab. Invest., 12: 737-747.

Bagai, R.C. & Subrahmanyam, D., 1970. Nature of acquired resistance to filarial infection in albino rats. Nature, 223: 682-683.

Basten, A. & Beeson, P.B., 1970. Mechanism of eosinophils. II. Role of the lymphocyte. J. exp. Med., 131: 1288-1305.

Basten, A., Boyer, M.H. & Beeson, P.B., 1970. Mechanism of eosinophilia. I. Factors affecting the eosinophil response of rats to Trichinella spiralis. J. exp. Med., 131: 1271-1287.

Buchanan, R.D., Fine, D.P. & Colley, D.G., 1973. Schistosoma mansoni infection in mice depleted of thymus-dependent lymphocytes. II. Pathology and altered pathogenesis. Amer. J. Pathol., 71: 207-214.

Capron, A., Capron, M., Camus, D. & Vernes, A., 1973. Les phénomènes d'hypersensibilité au cours des schistosomiases humaines à «Schistosoma mansoni» et «Shistosoma haematobium». II. Etude in vitro de l'activité léthale des sérums de malades pour les schistosomules de Schistosoma mansoni. Rapports avec les tests d'hypersensibilité. Pathologie-Biologie. 21: 1079-1084.

Clegg, J.A. & Smithers, S.R., 1972. The effects of immune rhesus monkey serum on schistosomula of Schistosoma mansoni during cultivation in vitro. Int. J. Parasitol. 2: 79-98.

Cohen, S., Rose, N.R. & Brown, R.C., 1974. The appearance of eosinophils during the development of experimental autoimmune thyroiditis in the guinea pig. Clin. Immunol. Immunopathol., 2: 256-265.

Cohen, B.S. & Ward, P.A., 1971. In vitro and in vivo activity of a lymphocyte and immune complex-dependent chemotactic factor for eosinophils. J. exp. Med., 133: 133-146.

Colley, D.G., 1971. Schistosomal egg antigen-induced lymphocyte blastogenesis in experimental murine Schistosoma mansoni infection. J. Immunol., 107: 1477-1480.

Colley, D.G., 1972a. Schistosoma mansoni: Eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. Exp. Parasitol., 32: 520-526.

Colley, D.G., 1972b. Intradermal immune responses to a schistosomal egg antigen during experimental murine Schistosoma mansoni infection. Proc. Soc. exp. Biol. Med., 140: 772-775.

Colley, D.G., 1973. Eosinophils and immune mechanisms. I. Eosinophil stimulation promoter (ESP): A lymphokine induced by specific antigen or phytohemagglutinin. J. Immunol. 110: 1419-1423.

Colley, D.G. Magalhaes-Filho, A. & Barros Coelho, R., 1972. Immunopathology of dermal reactions induced by Schistosoma mansoni cercariae and cercarial extract. Amer. J. trop. Med. Hyg., 21: 558-568.

Cotran, R.S. & Litt, M., 1969. The entry of granule-associated peroxidase into the phagocytic vacuoles of eosinophils. J. exp. Med., 129: 1291-1306.

Crowle, A.J., 1972. Trypsin-extracted immunizing antigen of the tubercle bacillus: A practical vaccine. Adv. Tuberc. Res., 18: 31-102.

Dean, D.A., Wistar, R. & Chen, P., 1975. Immune response of guinea pigs to Schistosoma mansoni. I. In vitro effects of antibody and neutrophils, eosinophils and macrophages on schistosomula. Amer. J. trop. Med. Hyg., 24: 74-82.

Dean, D.A., Wistar, R. & Murrell, D., 1974. Combined in vitro effects of rat antibody and neutrophilic leukocytes on schistosomula of Schistosoma mansoni. Amer. J. trop. Med. Hyg., 23: 420-428.

Dolovich, J., Hargreave, F.E., Chalmers, R., Shier, K.J., Gauldie, J. & Bienestock, J., 1973. Late cutaneous allergic responses in isolated IgE-dependent reactions. J. Allergy clin. Immunol., 52: 38-46.

Fine, D.P., Buchanan, R.D. & Colley, D.G., 1973. Schistosoma mansoni infection in mice depleted of thymus-dependent lymphocytes. I. Eosinophilia and immunologic responses to a schistosomal egg preparation. Amer. J. Pathol., 71: 193-206.

Hunter, G.W. III., Garcia, B.S., Crandall, R.B., Zickafoose, D.E. & Senterfitt, V., 1967. Studies on schistosomiasis. XXI. Attempts to enhance resistance to challenge infection by transfer of cells and parabiosis. J. Philippine med. Assoc., 43: 417-432.

Hsü, H.F. & Hsü, S.Y.L., 1974. Schistosomiasis in the Shanghai area. In: Quinn, J.R. (Ed.), China medicine as we saw it. DHEW Publ. No. (NIH) 75-684, pp. 345-363.

Hsü, S.Y.L. & Hsü, H.F., 1975a. A new hypothesis for the mechanism of immunity to schistosome infection. Fed. Proc., 34: 1026.

Hsü, S.Y.L. & Hsü, H.F., 1975b. Recovery of schistosomula in the skin of rhesus monkeys immunized with cercariae of Schistosoma japonicum exposed to high doses of X-irradiation. J. Parasitol., 61: 1108-1109.

Hsü, S.Y.L., Hsü, H.F. & Osborne, J.W., 1969. Immunization of rhesus monkeys against schistosome infection by cercariae exposed to high doses of X-irradiation. Proc. Soc. exp. Biol. Med., 131: 1146-1149.

Hsü, S.Y.L., Hsü, H.F., Penick, G.D., Lust, G.L. & Osborne, J.W., 1974. Dermal hypersensitivity to schistosome cercariae in rhesus monkeys during immunization and challenge. I. Complex hypersensitivity reactions of a well-immunized monkey during the challenge. J. Allergy clin. Immunol., 54: 339-349.

Hsü, S.Y.L., Hsü, H.F., Penick, G.D., Lust, G.L., Osborne, J.W. & Cheng, H.F., 1975. Mechanism of immunity to schistosomiasis: Histopathological study of lesions elicited in rhesus monkeys during immunizations and challenge with cercariae of Schistosoma japonicum. J. reticuloendothel. Soc., 18: 167-185.

Hsü, S.Y.L., Lust, G.L. & Hsü, H.F., 1971. The fate of challenge schistosome cercariae in a monkey immunized by cercariae exposed to high doses of X-irradiation. Proc. Soc. exp. Biol. Med., 136: 727-731.

Kaliner, M.A., Wasserman, S.I. & Austen, K.F., 1973. Immunologic release of chemical mediators from human nasal polyps. N. Engl. J. Med., 289: 277-281.

Keller, R. & Keist, R., 1972. Protective immunity to Nippostrongylus brasiliensis in the rat. Central role of the lymphocyte in worm expulsion. Immunology, 22: 767-773.

Khalil, M. & Hassan, A., 1932. A preliminary note on a new skin test in schistosomiasis. J. Egypt. med. Assoc., 15: 129-130.

Larsh, J.E., 1967. The present understanding of the mechanism of immunity to Trichinella spiralis. Amer. J. trop. Med. Hyg., 16: 123-132.

Larsh, J.E. Jr. & Weatherly, N.F., 1975. Cell-mediated immunity against parasitic worms. Adv. Parasitol., 13: 182-222.

Leber, P.D., Milgrom, M. & Cohen, S., 1973. Eosinophils in delayed hypersensitivity skin reaction sites. Immunol. Comm., 2: 615-620.

Long, B.Z., 1967. Host-parasite relationships of Fasciola hepatica in the white mouse. II. Studies on acquired immunity. J. Parasitol., 53: 21-30.

Maddison, S.E., Geiger, S.J., Botero, B. & Kagan, I.G., 1970. Schistosoma mansoni infection in the rat. II. Effects of immunosuppression and serum and cell transfer on innate and acquired immunity. J. Parasitol., 56: 1066-1073.

Maddison, S.E., Hicklin, M.D., Conway, B.P. & Kagan, I.G., 1972. Transfer factor: delayed hypersensitivity to Schistosoma mansoni and tuberculin in Macaca mulatta. Science, 173: 757-758.

Maddison, S.E., Hicklin, M.D. & Kagan, I.D., 1973. Immediate, Arthus, and delayed-type skin reactions in rhesus monkeys infected with Schistosoma mansoni or mycobacteria. J. Allergy clin. Immunol., 52: 131-140.

Miller, T.A., 1967. Transfer of immunity to Ancylostoma caninum infection in pups by serum and lymphoid cells. Immunology, 12: 231-241.

Moriearty, P.L. & Lewert, R.M., 1974a. Delayed hypersensitivity in Ugandan schistosomiasis. I. Sensitivity, specificity, and immunological features of intradermal responses. Amer. J. trop. Med. Hyg., 23: 169-178.

Moriearty, P.L. & Lewert, R.M., 1974b. Delayed hypersensitivity in Ugandan schistosomiasis. II. Epidemiological patterns of intradermal responses. Amer. J. trop. Med. Hyg., 23: 179-189.

Moriearty, P.L. & Lewert, R.M., 1974c. Delayed hypersensitivity in Ugandan schistosomiasis. III. Examination of serological responses and clinical states. Amer. J. trop. Med. Hyg., 23: 190-196.

Murrell, K.D. & Clay, B., 1972. In vitro detection of cytotoxic antibodies to Schistosoma mansoni schistosomules. Amer. J. trop. Med. Hyg., 21: 569-577.

Newsome, J., 1962. Immune opsonins in schistosome infestations. Nature, 195: 1175-1179.

Okamoto, K. & Kiozumi, M., 1972. Hymenolepis nana: effect of antilymphocyte serum on acquired immunity in mice. Exp. Parasitol., 32: 56-61.

Pellegrino, J. & Memoria, J.M.P., 1960. The skin test in schistosomiasis mansoni. V. Delayed type of cutaneous responses. Rev. Inst. Med. Trop. São Paulo, 2: 265-267.

Perez, H., Clegg, J.A. & Smithers, S.R., 1974. Acquired immunity to Schistosoma mansoni in the rat: measurement of immunity by the lung recovery technique. Parasitology, 69: 349-359.

Philips, S.M., Reid, W.A., Bruce, J.I., Hedlund, K., Colvin, R.C., Campbell, R., Diggs, C.L. & Sadun, E.H., 1975. The cellular and humoral immune response to Schistosoma mansoni infections in inbred rats. I. Mechanisms during initial exposure. Cell. Immunol., 19: 99-116.

Rothwell, T.L. & Dineen, J.K., 1972. Cellular reactions in guinea-pigs following primary and challenge infection with Trichostrongylus colubriformis with special reference to the roles played by eosinophils and basophils in rejection of the parasite. Immunology, 22: 733-745.

Rothwell, T.L.W., Dineen, J.K. & Love, R.G., 1971. The role of pharmacologically-active amines in resistance to Trichostrongylus colubriformis in the guinea-pig. Immunology, 21: 925-938.

Sadun, E.H. & Biocca, E., 1962. Intradermal and fluorescent antibody tests on humans exposed to Schistosoma bovis cercariae from Sardinia. Bull. Wld Hlth Org., 27: 810-814.

Sher, A., Kusel, J.R., Perez, H. & Clegg, J.A., 1974. Partial isolation of a membrane antigen which induces the formation of antibodies lethal to schistosomes cultured in vitro. Clin. exp. Immunol., 18: 357-369.

Sher, A., Smithers, S.R. & Mackenzie, P. 1975. Passive transfer of acquired resistance to Schistosoma mansoni in laboratory mice. Parasitology, 70: 347-357.

Smith, M. & Webbe, G., 1974. Damage to schistosomula of Schistosoma haematobium in vitro by immune baboon and human sera and absence of cross reaction with Schistosoma mansoni. Trans. roy. Soc. trop. Med. Hyg., 68: 70-71.

Smithers, S.R. & Terry, R.J., 1969. The immunology of schistosomiasis. Adv. Parasitol., 7: 41-92.

Torisu, M., Yoshida, T., Ward, P.A. & Cohen, B.S., 1973. Lymphocyte-derived eosinophil chemotactic factor. II. Studies on the mechanism of activation of the precursor substance by immune complexes. J. Immunol., 111: 1450-1458.

Vernes, A., Floc'h, F. & Biguet, J., 1972a. L'hypersensibilité de type retardé au cours de la bilharziose experimentale à Schistosoma mansoni. I. Etude in vitro chez la souris CBA. Ann. Inst. Pasteur, 123: 707-720.

Vernes, A., Biguet, J. & Floc'h, F., 1972b. L'hypersensibilité de type retardé au cours de la bilharziose experimentale due à Schistosoma mansoni. II. Etude in vitro chez le rat et le hamster doré. Comparaison avec la souris et résultats en fonction de l'adaption parasitaire. Ann. Inst. Pasteur, 123: 721-730.

Vernes, A., Biguet, J., Floc'h, F. & Lefebvre, M.N., 1972c. L'hypersensibilité de type retardé au cours des helminthiases expérimentales: étude du phénomène chez le cobaye in vivo et in vitro. Résultats préliminaires chez l'homme. Pathol. Biol., 20: 23-29.

Vernes, A., Camus, D., Capron, A., Capron, M. & Gentilini, M., 1973. Les phénomènes d'hypersensibilité au cours des schistosomiases humaines à «Schistosoma mansoni» et «Schistosoma haematobium». I. Etude de l'hypersensibilité retardée in vivo par intradermo-réaction et in vitro par le test de migration leucocytaire et un test mixte dérivé au test d'inhibition de l'étalement des macrophages. Pathologie-Biologie, 21: 1073-1078.

Wagland, B.M. & Dineen, J.K., 1965. The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of guinea-pig. Austral. J. expl. Biol. Med. Sci., 43: 429-438.

Warren, K.S., 1972. The immunopathogenesis of schistosomiasis: a multidisciplinary approach. Trans. roy. Soc. trop. Med. Hyg., 66: 417-434.

Warren, K.S., Kellermeyer, R.W., Jordan, P., Littell, A.S., Cook, J.A. & Kagan, I.G., 1973. Immunologic diagnosis of schistosomiasis. I. A controlled study of intradermal (immediate and delayed) and serologic tests in St. Lucians infected with Schistosoma mansoni and in uninfected St. Vincentians. Amer. J. trop. Med. Hyg., 22: 189-198.

Wasserman, S.I., Goetzl, E.J. & Austen, K.F., 1974. Preformed eosinophil chemotactic factor of anaphylaxis. J. Immunol., 112: 351-358.

Wolfson, R.L., Horner, D.W. & Kagan, I.G., 1969. Delayed skin sensitivity in schistosomiasis. J. Parasitol., 55: 1174-1179.

Wolfson, R.L., Maddison, S.E. & Kagan, I.G., 1972. Migration inhibition of peripheral leukocytes in human schistosomiasis. J. Immunol., 109: 123-128.

World Health Organization, 1973. Cell-mediated immunity and resistance to infection. WHO Techn. Rep., Ser. No. 519: 6-64.

LABELED ANTI-GLOBULIN ASSAYS FOR THE STUDY OF
SCHISTOSOME ANTIGENS AND HUMAN IMMUNE RESPONSES*

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For some time we have been using the indirect haemagglutination (IHA) and indirect fluorescent antibody (IFA) techniques for schistosome serological testing in our laboratories. Recently, however, we have found these test procedures somewhat difficult to adapt to newer experimental problems and even more difficult to quantitate precisely in these newer situations. For these reasons we have turned to three other labeled anti-immunoglobulin assays in order to take advantage of their highly quantitative and adaptable nature.

We first adapted an enzyme-linked immunosorbent assay (ELISA) to our schistosome system. Buffer containing only 1 μg of adult *Schistosoma mansoni* freeze-thaw antigen protein was used to sensitize plastic tubes. After an overnight incubation, these tubes were washed and incubated for 2-hr with 1 ml of a 1:400 dilution of patient serum, or the proper controls. If the specific anti-schistosome antibodies were present, they bound to the antigen on the inner surface of the plastic tube. A second 2-hr incubation

with goat anti-human IgG labeled with alkaline phosphatase enzyme was then required. If patient antibody was bound to the antigen, the enzyme-labeled goat anti-human antibody would in turn bind to it. A final 15 min. incubation with buffer containing 1 mg of the p-nitrophenylphosphate substrate was used.

As enzyme reacted with the substrate, a yellow color was formed which was visible to the unaided eye and which could be precisely measured by a spectrophotometer at 400 nm. The yellow color was a precise indication of the amount of enzyme present and indirectly a measure of the patient antibody.

A radioimmunoassay (RIA) was also adapted using the same tube bound antigen. In this procedure, goat anti-human IgG labeled with ^{125}I was used as the indicator. The test was performed in essentially the same manner as the ELISA technique, without the final substrate incubation. The amount of ^{125}I indicator present was determined by 1 min. counts in a gamma counter.

A third methodology utilized the same freeze-thaw adult worm antigen bound to microcrystalline cellulose particles by the cyanogen bromide technique. This third technique was also a radioimmunoassay using ^{125}I. The test was performed in tubes in a similar manner, but required that the microcrystalline cellulose particles be separated by centrifugation after each washing.

These labeled anti-globulin methods have shown great promise as research tools in our laboratory and are currently being used to assist in the identification of specific schistosome antigens which elicit an immune response in the human host. We have also begun to compare these methods with the indirect hemagglutination (IHA) and indirect fluorescent antibody (IFA) techniques as screening methods for epidemiological and clinical specimens.

In a comparison of quantitative results obtained with the five serological methodologies, 15 Egyptian *Schistosoma mansoni* patient serum specimens were studied. Spearman's Ranking Test was used to determine the amount of correlation in quantitative results. If high and low test results were perfectly matched in the two methods compared, a value of 1 would be obtained. Fractional values indicated lesser degrees of correlation and negative numbers indicated an inverse correlation.

The best correlation in quantitative results (0.94) was found between the ELISA assay and the RIA technique using the identical tube bound antigen. The poorest correlation in serological results (—0.19 to 0.16) was found between the IFA test and other serological methods. This we believe was due to the use of whole fixed cercariae in the IFA test, while the other methods used soluble

adult worm freeze-thaw antigen bound to some substrate.

When egg count data were compared with serological results, they were found to give a poor quantitative correlation with serological findings. The technique giving the best agreement (0.57) was the RIA using particle bound antigen.

In order to obtain a measure of the relative sensitivities of the five serological methodologies, a subset of eight *S. mansoni* patient sera were titrated to endpoint in each technique. It was found that the endpoint titers obtained with the newly adapted labeled anti-globulin assays were up to 10-fold higher than those obtained with the IHA or IFA tests. The one exception was a single specimen which gave its highest titer in the IHA test. It was also interesting to note that five of the eight sera titrated gave identical endpoints in all three of the newly adapted labeled anti-globulin assays.

In a preliminary study of the specificity of the ELISA and RIA techniques, 20 specimens from Egyptians passing only *S. mansoni* eggs, 10 from Egyptians passing only *S. haematobium* eggs, and 10 from normal blood bank donors, were tested by each method. It should be pointed out at this point that, while only one species of egg was being passed by these patients, it was impossible to rule out an earlier exposure to the other species.

It was found that *S. haematobium* patient results generally fell between those of *S. mansoni* patients and normal blood bank donors. As these results were obtained using only *S. mansoni* antigen, it was not possible to determine if this apparent difference was qualitative or quantitative in nature. However, in some recently obtained preliminary results using both *S. mansoni* and *S. haematobium* antigens, the relative levels of re-

sults were not substantially changed whether the immune serum and antigen used were homologous or heterologous. The *S. mansoni* patient findings remained generally higher with both antigens.

Very little testing has been done to date with sera from other helminth infections, but no cross reactions were seen using six sera from patients with *Trichinella spiralis* infections. Other possible cross reactions will be tested as appropriate sera become available.

The value of the newly adapted labeled anti-globulin assays is yet to be fully tested in clinical and epidemiological studies. They may often vary in usefulness depending on the circumstances under which they are to be used. While both the enzyme and isotope linked assays appear to be highly sensitive and very easy to quantitate, much will depend on the availability of reagents and specialized equipment necessary for their use. The need will also remain, as in other serological testing, for purified specific antigens which will produce serological data that correlate with clinical findings. Several antigen preparation and fractionation procedures are underway in our laboratories and these antigens will be investigated as part of an ongoing study of an Egyptian population at risk to schistosome infection.

SCHISTOSOME ALLERGENS

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Helminth infections are frequently associated with atopic hypersensitivity reactions and elevated IgE antibody levels (Sadun, 1972). A number of studies with purified helminth allergens have been reported as for example, work with ascaris allergens (Hussain et al., 1972, 1973), *Nippostrongylus brasiliensis* (Ambler & Orr, 1972) and schistosomes (Williams et al., 1965 ; Sato et al., 1969 ; Harris, 1973, 1975).

During the past few years we have been interested in the role of the reagin immune response in schistosomiasis. In the course of this work, attention has been given to the isolation and characterization of allergens from schistosomes (Vannier et al., 1974 ; Hussain et al., 1975). In most cases, fractions have been extracted and purified from adult *Schistosoma mansoni* and the allergen activity determined by Prausnitz-Kustner (P-K) serum passive transfer assays using serum from infected rats.

We have used a wide variety of methods (Table 1) for the extraction and solubilization of antigens from adult schistosomes. Many of these experiments are based on the application of methods that have been used to extract and solubilize cell membrane materials. The antigen fractions obtained by these methods were assayed for allergen activity by P-K assay with serum from rats with either single or multiple schistosome infections. A P-K assay with a freeze-thaw extract (Sch. A) is shown in Fig. 1. The allergen activity is measured by the difference in mean diameters between the reactions at skin sites sensitized with a dilution of rat infection serum and the same dilution of normal rat serum. In most cases, the sites were challenged with 1 μg of allergen fraction as measured by the Lowry method (Lowry et al., 1951) using a bovine plasma albumin standard. Table 2 lists the various fractions in order of allergen reactivity as shown by P-K testing with sera from rats with a single infection and rats with four infections. In each case, the same amount of allergen fraction (1 μg) was used for challenge. It is particularly striking that the least

active fraction (Sch. G), when tested with the single infection sera, became the most active fraction when tested with the serum pool from the multiply infected rats. In general, our data suggest that multiple allergens are involved in the reagin immune response in experimental rat schistosome infections.

In another experiment, a group of adult antigen fractions, cercarial fractions and a soluble egg antigen were assayed for allergen activity by the P-K

procedure using serum from rats immunized with the freeze-thaw antigen (FT-1) as well as serum from infected rats. The data are presented in Table 3. The adult antigens were obtained by freeze-thaw extraction (FT-1 and FT 2-6), by disruption with a French pressure cell, by recovery from the media after the worms had been in tissue culture for a few days (culture antigen) or storage in buffered saline for about 72 hr at 4°C (adult exoantigen). The cercarial exoantigen was prepared in a manner

TABLE 1. Antigen fractions from adult Schistosoma mansoni*

Fraction	Description**	Yield (mg/100,000 worms)
Sch. A	Freeze-thaw (6 cycles)	890
Sch. B	Homogenate of washed freeze-thaw residue	18
Sch. C	Sodium dodecylsulfate solubilized fraction of washed homogenate residue	337
Sch. D	Succinylation solubilized fraction of washed homogenate residue	200
Sch. Es	Triton X-100 solubilized fraction of washed homogenate residue . . .	316
Sch. Er	Saline soluble fraction of the Triton X-100 insoluble fraction	14
Sch. F	Normal butanol solubilized fraction of washed Triton X-100 residue	9
Sch. G	Fraction from homogenate residue solubilized by mercaptoethanol reduction and iodoacetate alkylation	120
Sch. H	Potassium thiocyanate (3M) solubilized fraction from homogenate residue	69
Sch. I	Fraction from homogenate residue solubilized by complex solvent***	225
XV	Buffer extract of lyophilized, lipid extracted whole worms	120
3M KCl	Potassium chloride (3M) extract of fresh adult worms	160-200
Cercarial enzyme	Enzyme fraction obtained by skin lipid stimulation of fresh cercariae	—

* Data from Vannier et al. (1974).

** The fractions are the pH 7.9 borate-saline soluble materials obtained by the various methods.

*** The composition of the complex extraction solvent is as follows: 2% sodium dodecylsulfate, 5M urea, 1% mercaptoethanol and 0.01% ethylenediaminetetraacetic acid in pH 7.9 borate saline.

analogous to the adult exoantigen and the soluble egg antigen by the method of Boros & Warren (1970). It is striking that the serum from infected rats gave more intense P-K reactions with cercarial antigens than the serum from rats immunized with the adult freeze-thaw antigens. In contrast, the immunization rat serum gave larger reactions with soluble egg antigen than did the infection serum.

This is entirely consistent with expectation since the infected rats were exposed to cercariae but not egg antigens and the immunized rats would never have had contact with cercariae but were immunized with the freeze-thaw fraction from adult worms containing eggs. This consistency suggests that the reactions we are studying are related in a direct way to the biology of schistosomiasis.
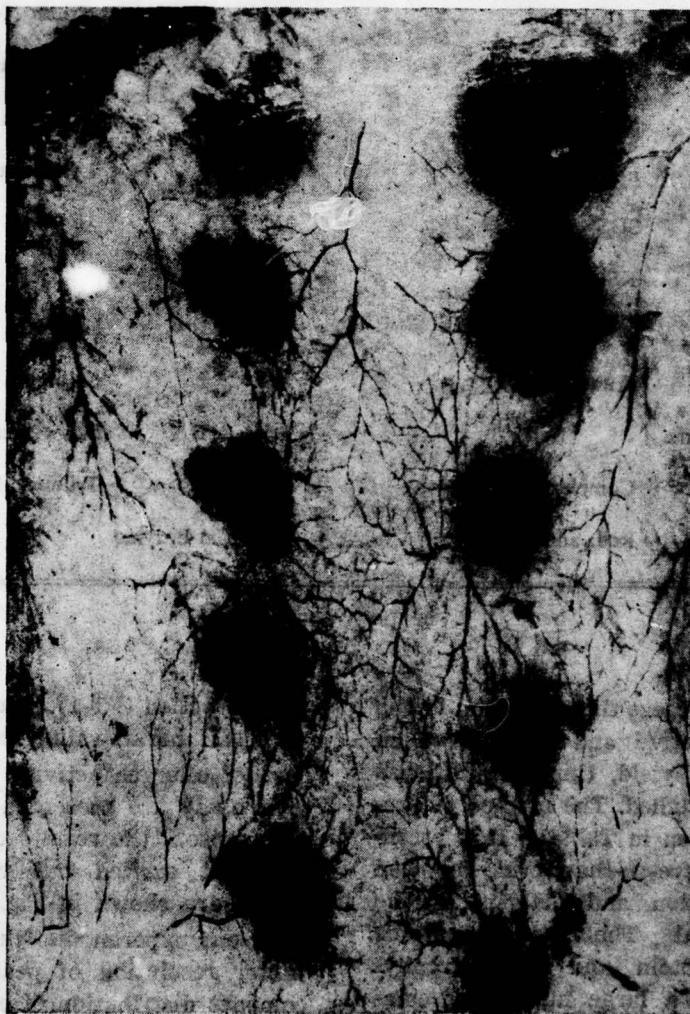
Fig. 1. P-K titration with freeze-thaw antigen and rat infection serum (right column) and normal rat serum (left column). The spots were challenged, starting at the top with 10 µg, 5 µg, 1.0 µg and 0.1 µg of FT-1 and the last site with 300 µg of human serum albumin. The differences in mean diameters between the test and normal serum sites reflect the strength of the reactions.

TABLE 2. Allergen activity by P-K assay with sera from *Schistosoma mansoni* infected rats

Single Infection (1000 Cercariae)			Multiple Infection (4 × 1000 Cercariae)		
Sch.	A	Freeze thaw	Sch.	G	Red. and Alk.
Sch.	B	Homogenate	Sch.	A	Freeze thaw
XVI		Lyophilized	Sch.	Er	Triton X-100
Sch.	Es	Triton X-100	Sch.	D	Succinic anhydride
Sch.	D	Succinic anhydride	Sch.	D	Succinic anhydride
Sch.	F	n-BuOH	XVI		Lyophilized
Sch.	C	SDS	3M KCl		
Sch.	H	KSCN	Sch.	F	n-BuOH
3M KCl			Sch.	H	KSCN
Sch.	Er	Triton X-100	Sch.	I	Solvent
Cercarial enzyme			Sch.	B	Homogenate
Sch.	I	Complex solvent	Sch.	Es	Triton X-100
Sch.	G	Red. and Alk.	Cercarial Enzyme		

Since the freeze-thaw fraction is one of the more active and is obtained in relatively good yield, this material has been further studied. The method of preparation is given in Table 4. It is essentially a single freeze-thaw cycle followed by concentration and dialysis of the soluble material. The fraction contains about 90% protein and 10% carbohydrate and has a large number of components as shown by disc and immunoelectrophoresis.

Sephadex G-100 gel filtration studies have been carried out with the freeze-thaw fraction (FT-1). The elution pattern in 0.17M borate-saline and the distribution of allergen activity as measured by P-K assay are presented in Fig. 2. For comparison, the results of a similar experiment carried out with 2M borate saline are shown in Fig. 3. It is clear from our experiments that we obtain a better resolution of peaks and a more compact distribution of allergen activity when the gel filtration is carried out in high salt buffer. The effect of the salt may be to decrease self-association or adsorption to the Sephadex.

TABLE 3. P-K assay of adult, cercarial and egg antigens with infection and immune serum*

Antigens	Infection Serum Diameter in mm	Immune Serum Diameter in mm
Adult Antigens:		
FT-1	8.0	5.0
FT 2- -6	7.5	3.0
French Press	5.0	3.0
Culture Antigen	6.5	3.5
Adult Exoantigen	6.0	4.0
Cercarial Antigens:		
Cercarial Homogenate	1.5	0
Cercarial Exoantigen	5.0	3.0
Cercarial Enzyme	3.0	0
Egg Antigens		
Soluble Egg Antigen	1.5	3.5
Diluent (HSA)	0.5-1.0	0-1.0

* Data from Hussain et al. (1975). P-K assays were carried out in normal rats with sera from *S. mansoni* infected rats (PCA titer with FT-1, 1 : 20) and rats immunized with FT-1 (PCA titer with FT-1, 1 : 40), 0.1 ml volumes of 1 : 4 dilutions of either sera were used for sensitization. Normal rat serum diluted 1 : 4 was used for antigen control reactions. Volumes of 0.1 ml containing 1.0 mg of the test antigen saline with 300 µg of pyrogen-free human serum albumin were used to challenge the skin sites. The results are expressed as the differences (mm) in skin reactions between the test and control sites. Each difference in diameter represents four replicate tests.

TABLE 4. Preparation of freeze-thaw antigen (FT-1) from *Schistosoma mansoni*

1. Swiss mice each infected with 200 cercariae.

2. Worms harvested by portal perfusion, washed, frozen and thawed once.

3. Worm supernatant concentrated, dialyzed and centrifuged. Supernatant designated FT-1.

4. FT-1 is a mixture of proteins and glycoproteins (90% protein and 10% carbohydrate).

SEPHADEX G-100 FRACTIONATION (0.15M BORATE SALINE)
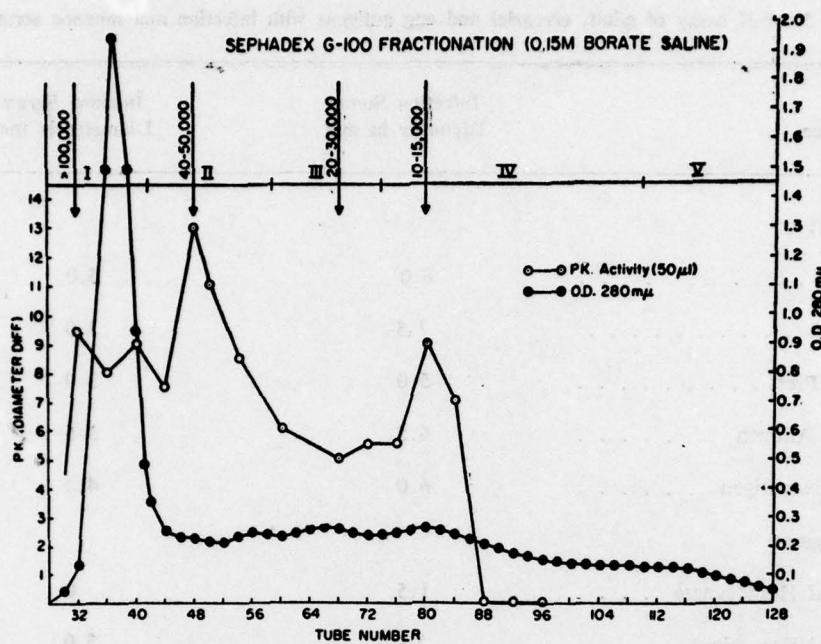
Fig. 2. Sephadex G-100 gel filtration fractionation of FT-1 in pH 7.9 borate-buffered saline (0.15M). The solid dots show the 280 nm absorbance elution profile and the open circles the P-K assay for allergen activity by challenge of the sensitized sites with 50 μl of eluate. The arrows indicate the position of reference protein markers.

SEPHADEX G-100 FRACTIONATION (2M BORATE SALINE)
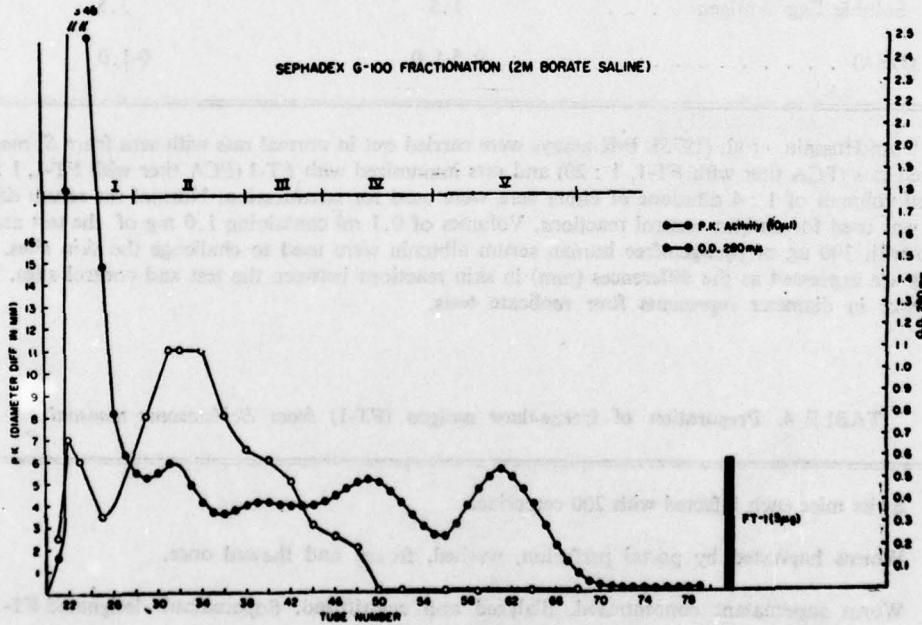
Fig. 3. Sephadex G-100 gel filtration of FT-1 in pH 7.9 borate-buffered saline (2M). The solid dots show the 280 nm absorbance elution profile and the open circles the P-K assay for allergen activity by challenge of the sensitized sites with 10 μl of eluate. The bar at the lower right indicates the P-K response to challenge with 5 μg of FT-1. The Roman numerals indicate fractions pooled for further study.

Table 5 shows the results of an assay of the immunogenicity, for reagin production, of the materials obtained by high salt gel filtration. It is clear that a broader range of materials will show allergen activity by reaction with sera from infected rats than will induce reagin formation on immunization of rats.

The peak 2 material, in the 50,000 molecular weight range, that we have shown to be immunogenic was further fractionated by isoelectric focussing methods. The results of such an experiment are presented in Fig. 4, and show two regions of allergen reactivity with pI's of approximately 4.8 and 6.2. When the disc electrophoresis patterns of G-100

peak 2 material are examined, two bands are seen near the anodal end of the gel (Fig. 5). When the gel was cut in 2 mm sections, eluted and the soluble material P-K tested, most of the P-K activity was in the region of the anodal bands. It is tempting to speculate that these bands represent the peaks of allergen activity seen in isoelectric focussing experiments.

The freeze-thaw extract has been fractionated with ammonium sulfate. Table 6 shows the yields of the fractions and the relative allergen activities on P-K assay with rat infection serum. Additional gel filtration and allergen testing studies have been carried out with these materials and indicate that most of the

TABLE 5. PCA titers with sera from rats immunized* with G-100 gel filtration fractions
(2 M borate saline, pH 7.9)

Immunizing Antigen	Rat Number	PCA Titers	
		Challenge with 1 mg FT-1	Challenge with 1 mg Immunizing Antigen
G-100-I	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
G-100-II	1	1	1
	2	16	16
	3	64	64
	4	8	8
	5	32	32
G-100-III	1	8	16
	2	1	1
	3	16	16
	4	8	8
	5	0	0
Infection Serum Control**		20	

* Rats given 1 mg antigen S.Q. with 2×10^{10} *Bordetella pertussis* organism IP and bled in two weeks.

** Serum pool from rats infected twice with 2000-4000 *Schistosoma mansoni* cercariae.

FRACTIONATION OF G100 II BY ISO-ELECTRIC
FOCUSSING (pH 3-10)

○—○ P.K. 50µl
△—△ pH GRADIENT
●—● O.D. 280nm

BLANK RUN
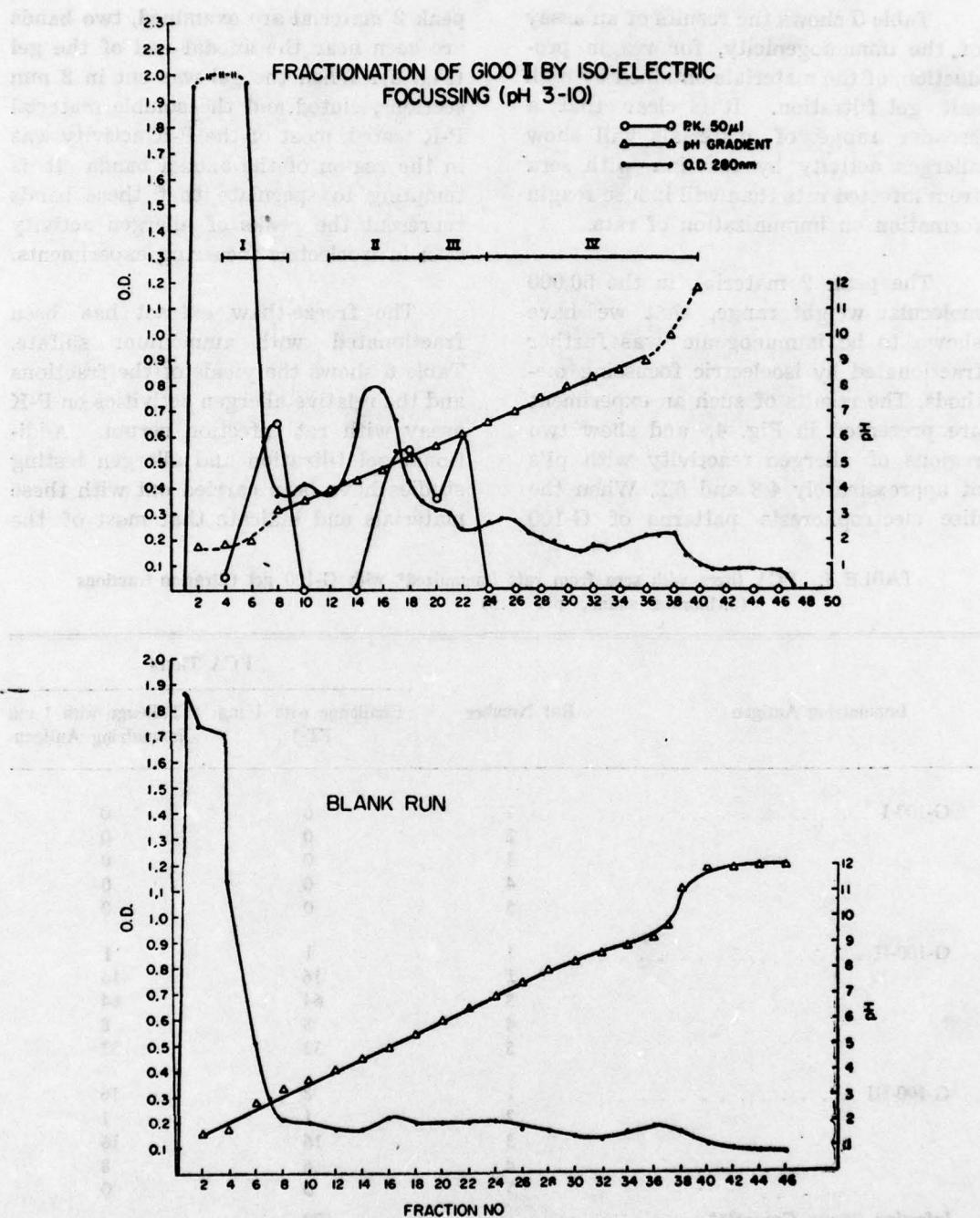
FRACTION NO

Fig. 4. Isoelectric focussing fractionation (pH gradient 3-10) of the 50,000 molecular
weight fraction from Sephadex G-100 gel filtration (G-100 II, 2M borate saline,
pH 7.9). The lower curve shows the absorbance elution profile from a blank run and
indicates the extent of the absorbance contribution by the amphyolytes used in the
isoelectric focussing.

Fig. 5. Acrylamide geldisc electrophoresis pattern of the 50,000 molecular weight fraction from Sephadex G-100 gel filtration (G-100 II, 0.15 M borate saline, pH 7.9). The anode is towards the bottom (here left side) of the disc gel.

50,000 molecular weight allergen activity is in the P50-60 and S60 fractions and that the higher molecular weight allergen is found in all fractions but probably in largest amount in the 40% ammonium sulfate precipitate.

Table 7 presents a summary of the allergen studies with the freeze-thaw extract. There is evidence for at least three allergens with different physical properties. One of them is of high molecular weight (200,000-500,000) and the other two are of about 50,000 molecular weight but have different isoelectric points (PI 4.8 and 6.2).

We are continuing our efforts to purify and further characterize schistosome allergens and to define their role in the induction of resistance to infection. Animals have not been immunized, as yet, with highly purified allergens for protection experiments ; however, as Dr. Murrell reported at an earlier session, reagin antibodies induced in mice with the freeze-thaw antigen (FT-1) may in conjunction with antibodies found in chronic infection mouse serum be involved in mediating protection against infection. Purified schistosome allergens may also be of interest for serodiagnostic methods in human disease.

TABLE 6. Ammonium sulfate fractionation of freeze-thaw antigen
(71 mg of FT-1)

Fraction		Yield, mg	P-K Assay (1 μg)
Precipitate 40% saturation	(P40)	21	+++
Precipitate 40-50% saturation	(P40-50)	4	+
Precipitate 50-60% saturation	(P50-60)	8	+
Supernatant 50% saturation	(S60)	15	+++

Yield 48 mg (68% recovery)

TABLE 7.   Freeze-thaw allergen fraction (FT-1) from adult *Schistosoma mansoni*

1.   90% protein; 10% carbohydrate

2.   Six to seven major bands acrylamide disc electrophoresis

       Six to seven major bands immunoelectrophoresis (rabbit antiserum against adult worms)

3.   Gel Filtration Studies:

       a)   200,000-500,000 MW fraction allergenic

       b)   50,000 MW fraction allergenic and immunogenic.

            Isoelectric focussing (50,000 MW fraction) shows two peaks of allergen activity (pI 4.8 and 6.2)

LITERATURE CITED

Ambler, J. & Orr, T.S.C., 1972. Studies on an allergenic component extracted from N. brasiliensis. Immunochem., 9: 263.

Boros, D.L. & Warren, K.S., 1970. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from Schistosoma mansoni eggs. J. exp. Med., 132: 488.

Harris, W.G., 1973. The allergens of Schistosoma mansoni. I. Initial separation and comparative assay. Immunol., 24: 267.

Harris, W.G., 1975. The allergens of Schistosoma mansoni. II. Further separation by Sephadex G-200 and ion-exchange chromatography. Immunol., 29: 835.

Hussain, R., Bradburry, S. & Strejan, G., 1973. Hypersensitivity to ascaris antigens. VIII. Characterization of a highly purified allergen. J. Immunol., 111: 260.

Hussain, R., Strejan, G. & Campbell, D.H., 1972. Hypersensitivity to ascaris antigens. VII. Isolation and partial characterization of an allergen. J. Immunol., 109: 638.

Hussain, R., Vannier, W.E. & Murrell, K.D., 1975. Hypersensitivity to Schistosoma mansoni anti-

gens. I. Immunochemical and biological characterization of an antigenic extract. Immunochem., 12: 561.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randall, R.J., 1951. Protein measurement with the Follin phenol reagent. J. biol. Chem., 193: 265.

Sadun, E.H., 1972. Homocytotropic antibody response to parasitic infections. In: Soulsby, E.J. (Ed.), «Immunity to Animal Parasites». Academic Press, New York, p. 104.

Sato, K., Sawada, T. & Sato, S., 1969. Studies on the purification of skin test antigen SST for the diagnosis of schistosomiasis japonica. II. The purification of antigen SST by disc-Sephadex G-25 column electrophoresis and disc electrophoresis. Jap. J. exp. Med., 39: 347.

Vannier, W.E., Fireman, B.I., Chestnut, R.Y. & Murrell, K.D., 1974. Schistosoma mansoni: Immediate hypersensitivity to adult antigens in the rat. Exp. Parasit., 36: 45.

Williams, J.E., Moose, J.W., Sawada, T., Takei, K. & Sato, S., 1965. Studies on the immunodiagnosis of schistosomiasis. I. Intradermal skin tests with fractionated antigens., J. inf. Dis., 115: 382.

PLACE ET ROLE DE L'HYPERSENSIBILITE
RETARDEE AU COURS DES BILHARZIOSES

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Que la démonstration en soit apportée *in vivo* ou *in vitro* et malgré la multiplication des travaux sur ce sujet depuis quelques années, il est difficile d'apprécier le rôle précis de l'hypersensibilité retardée (H.S.R.) dans l'immunité de protection vis-à-vis des schistosomes.

S'il a été démontré par différents auteurs, tant chez l'homme que dans les modèles expérimentaux, que l'H.S.R. était un phénomène assez banal au cours des schistosomiases, sa fréquence n'en subit pas moins des variations très sensibles d'un modèle à l'autre.

Le rôle joué par l'H.S.R. dans les bilharzioses est attesté par les expériences d'immunodépression entraînant une susceptibilité accrue à l'infection ou conduisant à une évolution plus sévère de la maladie expérimentale, de tels résultats élargissant le cadre de l'H.S.R. à celui de l'immunopathologie des schistosomiases.

Parmi les différents facteurs entrant en jeu dans l'appréciation du rôle de l'H.S.R. nous aimerions attirer l'attention sur deux d'entre eux :

1) L'intervention des antigènes communs hôte-parasite ;

2) Les phénomènes d'adaptation parasitaire.

1) Comme le montre le tableau 1 les antigènes communs à l'hôte intermédiaire *Biomphalaria glabrata* et au parasite *Schistosoma mansoni* participent *pro parte* à l'induction de l'H.S.R. chez le cobaye immunisé passivement ou chez la souris infestée et développant activement son H.S.R. Les résultats *in vitro* ici présentés sont retrouvés *in vivo* chez les mêmes animaux par intradermo-réaction.

2) Le deuxième facteur, l'adaptation parasitaire, représente à nos yeux une donnée importante dans l'appréciation de l'H.S.R. au cours des schistosomiases. Le degré d'H.S.R. observé est inversement proportionnel au degré de l'adaptation parasitaire :

a) chez les rongeurs expérimentalement infestés (Vernes et coll., 1972),

b) chez l'homme lorsque l'on compare la fréquence de l'H.S.R. chez des malades atteints de bilharziose intestinale ou de bilharziose à *S. haematobium* (Vernes et coll., 1973). Il faut noter également dans ce cadre humain que l'H.S.R. est plus fréquente dans les formes hépatospléniques de la schistosomiase à *S. mansoni* que dans les formes asymptômatiques de cette même parasitose (Camus, 1974).

Les résultats brièvement rapportés ici ne représentent qu'une infime partie de la contribution apportée ces dernières années par différentes équipes mais nous autorisent à attirer l'attention sur les deux points suivants :

TABLEAU 1. Etude des communautés antigéniques hôte-parasite par les tests *in vitro* d'hypersensibilité retardée.

Animaux	Nombre	Index de migration des macrophages (Cobayes) Index d'étalement des macrophages (souris) en présence de l'antigène	
		Schistosoma mansoni	*Biomphalaria glabrata*
Cobayes témoins	16	105,5	99,7
Cobayes immunisés, *S. mansoni* . . .	11	28	57,1
Cobayes immunisés, *B. glabrata* . .	10	65,4	41,2
Souris témoins	27	91,2	99,8
Souris infestées, *S. mansoni*	17	48,1	64,1

1) Sur un plan pratique et diagnostique, il ne semble pas que les techniques de détection *in vivo* et *in vitro* de l'H.S.R. puissent, en dehors de l'utilisation d'antigènes spécifiques, remplacer ou concurrencer les techniques immunosérologiques actuellement employées, car tout en présentant des difficultés matérielles de réalisation, elles conservent les mêmes défauts de non-spécificité absolue et manquent de sensibilité.

2) Sur un plan prospectif, les relations existant entre H.S.R., l'adaptation parasitaire et la physiopathologie des formes graves des schistosomiases doivent inciter à la prudence dans la mise au point d'une immunoprotection artificielle qui ne serait pas forcément valable pour toutes les schistosomiases et qui, d'autre part, risquerait d'entraîner des effets secondaires indésirables.

LITTERATURE

Camus, D., 1974. Les phénomènes d'hypersensibilité retardée dans la schistosomiase humaine à S. mansoni. Thèse Médecine, Lille.

Vernes, A., Biguet, J. et Floc'h, F., 1972. L'hypersensibilité de type retardé au cours de la bilharziose expérimentale dire à Schistosoma mansoni. Ann. Inst. Past., Paris, 123: 707-730.

Vernes, A., Camus, D., Capron, M. et Gentilini, M., 1973. Les phénomènes au cours de schistosomiases humaines à Schistosoma mansoni et Schistosoma haematobium. I. Etude de l'hypersensibilité retardée in vivo par intradermo-réaction et in vitro par le test de migration leucocytaire et un test mixte dérivé du test d'inhibition de l'étalement des macrophages. Path. Biol., 21: 1073-1078.

PENETRATION ENZYMES OF *SCHISTOSOMA MANSONI* CERCARIAE*

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Establishment of a schistosomal infection is dependent in part upon successful penetration of the skin barrier by cercariae. Skin penetration is accompanied by release of preacetabular gland material which contains one or more proteolytic enzymes. We wished to investigate the hypothesis that antibodies to the enzyme-containing secretion material could prevent cercarial penetration of the host and/or development of schistosomiasis. This paper describes the results of those experiments.

Materials and Methods

Cercarial secretion material, or CSM, was obtained by stimulating cercariae to penetrate an impenetrable surface. Approximately 20-25 μg protein were obtained per 1000 cercariae. Cercarial secre-tion material is heterogeneous : polyacrylamide gel electrophoresis indicated 10-15 protein components and four components which stained for carbohydrate.

The general procedure for all immunization experiments was as follows : NIH/NMRI§ female mice, aged 6 weeks at the initiation of the experiment, were immunized with crude cercarial secretion material in varying protocols. Crude material was used since antibodies to non-enzymatic components were potentially protective. Control mice received adjuvant only. Following the immunization series, each mouse was challenged with 100 cercariae by tail immersion. Mice were perfused 7-8 weeks later and worms were counted. Sera tested for antibody were obtained from immunized or control mice on the day of cercarial challenge.

Results and Discussion

Table 1 shows the results of three representative immunization experiments. In the first experiment, each mouse received a total of 3 mg of CSM in five injections, over a 5-week period. Freund's adjuvant and the high dose of antigen favor IgG production and T cell activation. No difference in worm recovery was observed between immunized and non-immunized mice. In the next experiment, mice again received 3 mg CSM in five injections over a 5-week period, but here the adjuvant was alum. This protocol in general favors IgG production. Again, no

effect on worm recovery was observed. Both of these experiments have been repeated using slightly different time courses for immunization. The results were the same, i.e. no reduction in adult worm recovery was observed.

In the third experiment in Table 1, mice were injected three times at monthly intervals with 10 μg CSM adsorbed to alum. Such an immunization regime has been used in other systems to induce IgE production. Worm recoveries again showed no difference between immunized and control mice.

TABLE 1. Immunization of mice with multiple injections of cercarial secretion material (CSM)

Group, (No. mice)	Adjuvant	Total CSM injected	Number of challenge worms recovered $\bar{X} \pm SD$
1. Immunized . . . . (29)	Freund's	3.0 mg	14 $\pm$ 13
Control . . . . (20)	Freund's	—	16 $\pm$ 8
2. Immunized . . . . (10)	Alum	3.0 mg	25 $\pm$ 10
Control . . . . (5)	Alum	—	27 $\pm$ 13
3. Immunized . . . . (29)	Alum	0.03 mg	32 $\pm$ 13
Control . . . . (10)	Alum	—	30 $\pm$ 13

TABLE 2. Concurrent immunization of mice with cercarial secretion material (CSM) and adult worm freeze-thaw extract (FT)

Group, (No. Mice)	Total CSM injected (adjuvant)	Route of injection	Number of challenge worms recovered $\bar{X} \pm SD$
Immunized (22)	0.03 mg CSM (Alum)	IP	25 $\pm$ 11
	4.50 mg FT (Freund's)	SC	
Controls (15)	(Alum)	IP	27 $\pm$ 10
	(Freund's)	SC	

IP = intraperitoneal
SC = subcutaneous

—

We attempted also to immunize mice to two schistosomal antigens concurrently. The results are shown in Table 2. Mice were immunized with low dose CSM adsorbed to alum in a manner known to raise IgE antibodies. Concurrently, the mice were also given adult freeze-thaw (FT) antigen in a protocol used in our laboratory to induce IgG cytotoxic antibodies. Control mice received adjuvants but no antigen. Once again, however, no reduction in worm recovery resulted from the immunization procedure.

Thus, we present four representative experiments in which mice were immunized with cercarial secretion material. In none of the experiments did immunization afford any protection from penetration of cercariae and their subsequent development to adult worms.

The possibility existed, of course, that lack of protection was due to failure to induce antibody formation. This was not, however, the case. Both IgG and IgE antibodies could be detected depending on the immunization conditions used. Figure 1 shows examples of immunodiffusion bands obtained with three different sera. The antibodies thus visualized are most probably of the IgG class. A single light precipitin line was seen with sera from mice immunized with low dose CSM in alum; no cross reactivity with adult freeze-thaw antigen was observed. Concurrent immunization with CSM and FT antigens resulted in light precipitin bands against CSM and heavier bands against FT antigen. Finally, FT antigen alone induced antibodies which did not cross react with CSM.

The results of passive cutaneous anaphylaxis (PCA) assays of the same sera for IgE antibodies are given in Table 3. Sera were tested both neat and at 1:5 dilution against both FT and CSM anti-

Fig. 1. Immunodiffusion bands demonstrating the presence of antibodies in the sera of mice immunized with CSM.

CSM = cercarial secretion material (antigen)

aCSM = anti-cercarial secretion material antiserum

NMS = normal mouse serum

FT = freeze-thaw antigen

aFT/CSM = anti - freeze - thaw/anti-CSM antiserum

Adjuvant control aFT/CSM = adjuvant control for concurrent immunization with FT and CSM

aFT = anti-freeze-thaw antiserum

TABLE 3. Stimulation of IgE antibody in mice immunized with cercarial secretion material (CSM) alone or in combination with adult worm freeze-thaw (FT) antigen*

Serum	Dilution	Challenge antigen	
		CSM	FT
Normal mouse	Neat	0**	0
	1 : 5	0	0
Anti-CSM	Neat	N.D.	26.2
	1 : 5	46.9	7.1
Anti-FT	Neat	15.1	17.7
	1 : 5	0	0.7
Anti-FT/CSM	Neat	49.3	39.2
	1 : 5	5.3	6.1

* Serum assayed by PCA in rats   N. D. = not done
** Average radius² (cm²)

gens. Moderate induction of IgE antibodies resulted from low dose immunization with CSM. Immunization with FT antigen did not result in especially high production of reaginic antibodies. Unlike the precipitating antibodies just discussed, some cross reactivity was observed between CSM and FT antigens. The IgE antibody response to CSM was somewhat depressed in mice immunized simultaneously with FT antigen. This result is

not very surprising given current ideas regarding IgG suppression of IgE responses.

These experiments demonstrate that IgE and/or IgE antibodies can be induced by immunization of mice with CSM. Such antibodies to the cercarial secretion material are not, however, sufficient to protect against development of schistosomiasis.

THE EOSINOPHIL POLYMORPHONUCLEAR LEUKOCYTE
IN SCHISTOSOMIASIS : A REVIEW

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Eosinophilia has been associated with allergic reactions, helminth infections and neoplasms for almost a century, but as Zucker-Franklin (1974) commented in a recent review, «whether these cells fulfill a detrimental or beneficial function is still open to question». In comparison with knowledge of the function of other white cells, the role of the eosinophil remains largely unknown. In the past it was suggested that eosinophils break down histamine released in the tissues (Archer, 1970) and that they may be involved in the induction of antibody formation (Speirs, 1970). Only in recent years, however, through the almost explosive nature of the progress in biochemical and immunological research, has a significant understanding of eosinophils and their role emerged. Basic to the development of this newer knowledge were the availability of techniques for separating and purifying eosinophils of human (Day, 1970 ; Mahmoud et al., 1974a, b) and animal origin (Mahmoud et al., 1973 ; Gleich & Loegering, 1973 ; Gleich et al., 1975) to characterize these cells antigenically and to raise specific antisera against them (Mahmoud et al., 1974b ; Gleich et al., 1975 ; Mahmoud, in press).

This presentation will review only the newer knowledge concerning the eosi-

nophil polymorphonuclear leukocyte as it relates to schistosomiasis. Other recent publications have dealt with the subject of eosinophils and eosinophilia in general (Zucker-Franklin, 1974 ; Goetzl et al., 1975 ; Chasid et al., 1975).

Eosinophilia in Schistosomiasis

As early as the beginning of this century, two reports were published on the association of schistosomiasis haematobia with an increase in the peripheral blood eosinophils (Russel, 1902 ; Coles, 1902). Until recently, while there were several reports describing the eosinophilia seen in experimental and human schistosomiasis (Sorour, 1930 ; Abou-El Naga & Saleh, 1952 ; Dewitt, 1953 ; McMatton, 1967), there were no detailed data on the kinetics of the eosinophilic response or its relation to intensity and duration of infection. We have undertaken to quantitate peripheral as well as bone marrow eosinophilia during the course of schistosomiasis mansoni in mice (Mahmoud et al., 1975a). Eosinophils were increased in the peripheral blood and bone marrow of infected animals in two distinct waves. The first, which occurred 3 weeks after exposure to the cercariae, was relatively minor and was seen in the peri-

pheral blood only in the mice with the heavy worm burden ; eosinophilia occurred in the bone marrow, however, at all three levels of infection used (Fig. 1, Mahmoud et al., 1975a). During this early stage of the infection, the schistosomula are in the process of migration from the lung to the liver. By 4 weeks the schistosomula had all migrated into the intrahepatic portal venules and the peripheral as well as the bone marrow eosinophilia dropped to normal. Later in the course of infection, a second major wave of eosinophilia occurred coincident with the appearance of mature antigen-secreting schistosome eggs in the host tissues (Fig. 1). The relationship of this second wave of eosinophilia to the worm burden was also studied. Maximum response was obtained in relatively moderate infection (50 cercariae) while animals with heavy infections (200 cercariae) succumbed and could not be followed up for prolonged periods. Study of the effect of duration of infection was possible in animals with the lowest worm burdens (10 cercariae). The eosinophil response was most marked at 8-10 weeks, but by 18-20 weeks it declined, a phenomenon which appears to parallel the «spontaneous modulation» of the granulomatous response seen in chronically infected animals (Boros et al., 1975).

The effect of injection of isolated schistosome eggs or the soluble egg antigen (SEA) obtained from these eggs on the eosinophil counts was also studied in both our and Colley's laboratory (Colley, 1972 ; Mahmoud et al., 1975a). Injection of eggs or SEA at different sites in experimental animals showed the characteristics of primary and secondary immunological responses as seen in previous experiments with *Trichinella spiralis* larvae (Basten et al., 1970).

The eosinophil is a prominent cell in the host granulomatous response to the schistosome eggs. We have studied the cellular responses around eggs injected into the pulmonary microvasculature of mice. In unsensitized and egg-sensitized animals, eosinophils were first seen around the eggs at 96 and 24 hr respectively (Mahmoud et al., 1975a). When the granulomas were maximal in size, the eosinophils made up at least 50% of the lesion. In contrast, administration of anti-eosinophil serum ablated the eosinophils from the granulomatous response and considerably reduced its area. Absence of eosinophils from the lesions resulted in granuloma volumes of less than 1/10 those seen in control animals. While these experiments may help to reveal the role of eosinophils in the immunopathology of schistosomiasis ; it has recently been suggested, however, that these cells may also play a role in destruction of the schistosome egg (James & Colley, 1976).

Mechanisms of Eosinophilia in Schistosomiasis

The eosinophilic response in helminth infections has been suggested to be dependent on the integrity of the lymphocyte population of the host (Basten & Beeson, 1970). In schistosomiasis the relationship between eosinophilia and the lymphocyte population was studied by Colley (Fine et al., 1973). Animals that were deprived of the majority of their T-cell population failed to show the second major wave of eosinophilia. Furthermore, Colley in a series of experiments (Colley, 1973 ; Greene & Colley, 1974 ; 1975) has shown that eosinophil migration can be stimulated by a product of the interaction of sensitized lymph node cells and the specific antigen (SEA in this case). This lymphocyte-derived activity was called Eosinophil Stimula-
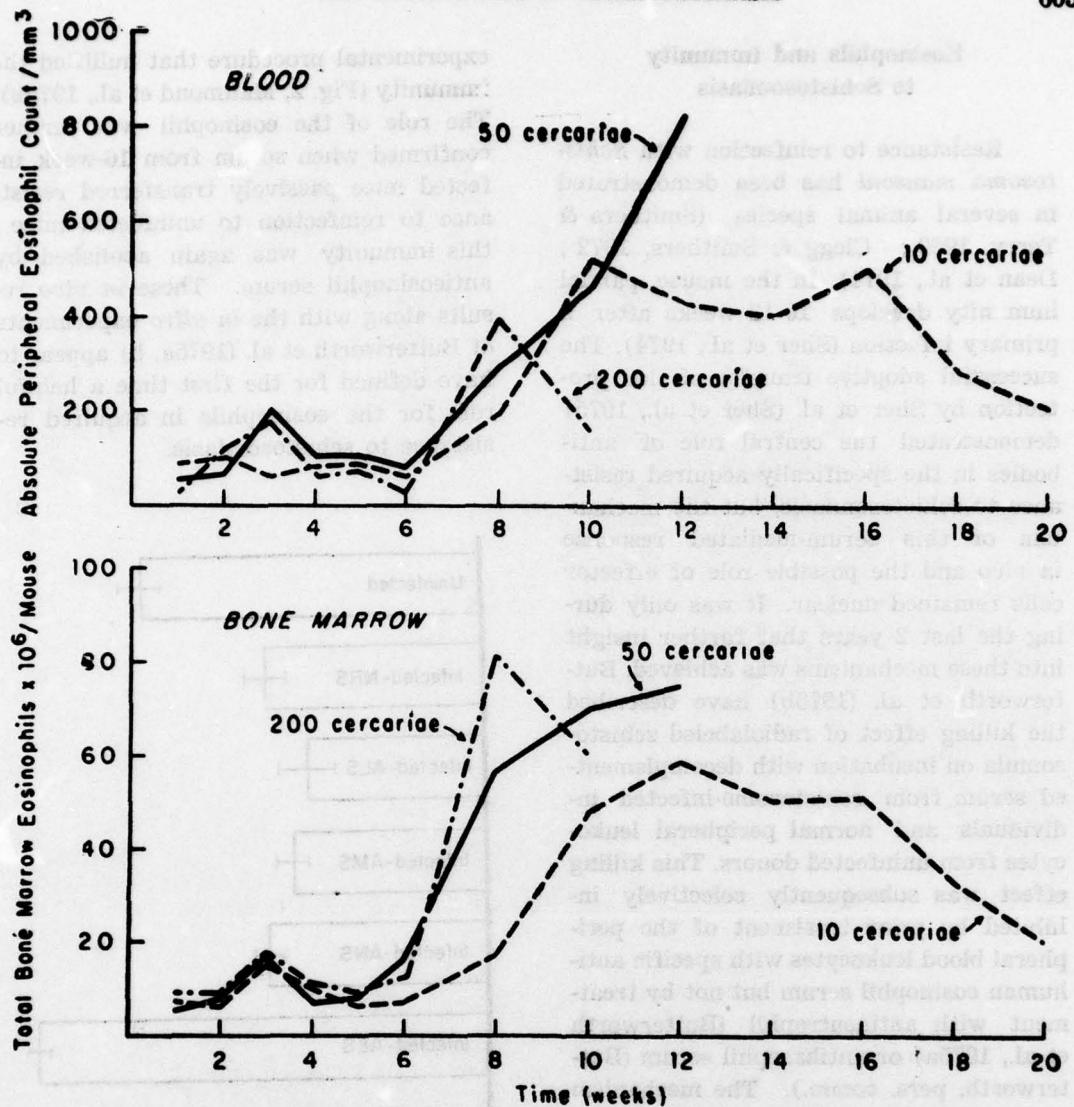
Fig. 1. Mean absolute peripheral blood eosinophil counts and total bone marrow eosinophils in mice exposed to 10, 50, and 200 cercariae of *Schistosoma mansoni*. Mortality prevented the follow up of the more heavily infected animals.

tion Promoter (ESP) and was later shown to be *de novo* synthesized protein with a molecular weight of 24,000-56,000 produced probably by T-lymphocytes. In recent studies in our laboratories (Pelley et al., in press), the ESP test has been shown to follow an antigen dose-dependent response curve similar to other lymphokine assays and to be antigen specific. At present two questions concerning ESP as a lymphokine need to be elucidated :

1) whether or not it is the same factor as MIF (Macrophage Migration Inhibitory Factor), and 2) what its chemotactic effect on the eosinophils may be. The usefulness of the ESP assay has recently been evaluated by adapting the system for human cells (Kazura et al., 1975), which may prove helpful for diagnostic as well as investigative purposes (Warren et al., in press).

Eosinophils and Immunity to Schistosomiasis

Resistance to reinfection with *Schistosoma mansoni* has been demonstrated in several animal species (Smithers & Terry, 1969; Clegg & Smithers, 1972; Dean et al., 1974). In the mouse, partial immunity develops 10-12 weeks after a primary infection (Sher et al., 1974). The successful adoptive transfer of this protection by Sher et al. (Sher et al., 1975) demonstrated the central role of antibodies in the specifically acquired resistance to schistosomiasis, but the mechanism of this serum-mediated response *in vivo* and the possible role of effector cells remained unclear. It was only during the last 2 years that further insight into these mechanisms was achieved. Butterworth et al. (1975b) have described the killing effect of radiolabeled schistosomula on incubation with decomplemented serum from schistosome-infected individuals and normal peripheral leukocytes from uninfected donors. This killing effect was subsequently selectively inhibited by prior treatment of the peripheral blood leukocytes with specific antihuman eosinophil serum but not by treatment with antineutrophil (Butterworth et al., 1975a) or antibasophil serum (Butterworth, pers. comm.). The mechanism of acquired immunity in infected mice was recently clarified by experiments performed in our laboratories (Mahmoud et al., 1975b). Partially immune animals as determined by a marked reduction in the numbers of schistosomula and adult worms on challenge with cercariae were exposed to antieosinophil serum. This treatment completely blocked the immunity of these animals. As a control for these experiments, we used animals pretreated with specific antineutrophil, antilymphocyte, or antimacrophage sera. Depletion of the eosinophil was the only

experimental procedure that nullified the immunity (Fig. 2, Mahmoud et al., 1975b). The role of the eosinophil was further confirmed when serum from 16-week infected mice passively transferred resistance to reinfection to uninfected mice; this immunity was again abolished by antieosinophil serum. These *in vivo* results along with the *in vitro* experiments of Butterworth et al. (1975a, b) appear to have defined for the first time a helpful role for the eosinophils in acquired resistance to schistosomiasis.

NUMBER OF SCHISTOSOMULA RECOVERED
FROM THE LUNGS
Mean ± S.E.
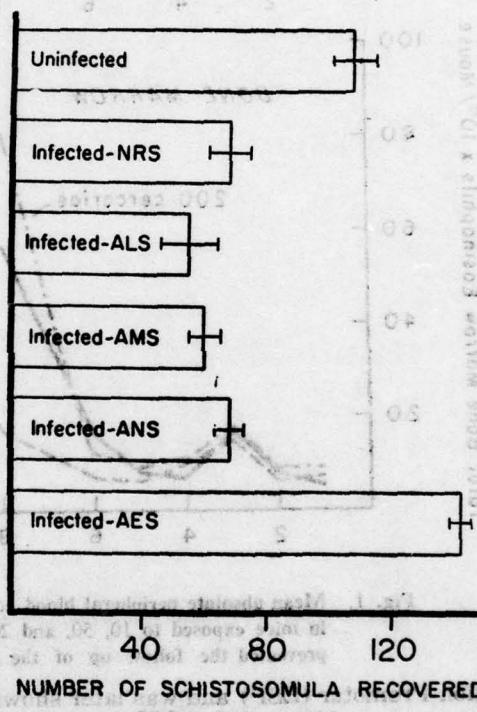
Fig. 2. Recovery of schistosomula 6 days after percutaneous exposure to 500 cercariae of *Schistosoma mansoni* of control, uninfected mice, and partially immune previously infected mice treated with normal rabbit serum (NRS) antilymphocyte serum (ALS), antimacrophage serum (AMS), antineutrophil serum (ANS), and antieosinophil serum (AES).

Conclusion

Aproximately 100 years after Ehrlich's pioneering work on the definition of the peripheral blood granulocytes, a definitive role for the eosinophils has been described. This knowledge should not only open the way to a better understanding of the function of eosinophils but may also help in the manipulation of the immune system of man for the purpose of increasing his resistance to one of the major helminth infections.

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LITERATURE CITED

Abou-El Naga, I. & Saleh, A., 1952. Blood eosinophilia in parasitic infections endemic in Egypt. J. Egypt. med. Assoc., 35: 526-532.

Archer, R.K., 1970. Regulatory mechanisms in eosinophil leukocyte production, release and distribution. In: Gordon, A.S. (Ed.), «Regulation of Hematopoiesis», Vol. 2. Appleton-Century-Crofts, New York, pp. 917-941.

Basten, A. & Beeson, P.B., 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. J. exp. Med., 131: 1288-1305.

Basten, A., Boyer, M.H. & Beeson, P.B., 1970. Mechanism of eosinophilia. I. Factors affecting the eosinophil response of rats to Trichinella spiralis. J. exp. Med., 131: 1271-1287.

Boros, D.L., Pelley, R.P. & Warren, K.S., 1975. Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. J. Immunol., 114: 1437-1441.

Butterworth, A.E., Sturrock, R.F., Houba, V., Mahmoud, A.A.F., Sher, A. & Rees, P.H., 1975a. Eosinophils as mediators of antibody-dependent damage to schistosomula. Nature (Lond.), 256: 727-729.

Butterworth, A.E., Sturrock, R.F., Houba, V. & Rees, P.H., 1975b. Antibody-dependent cell-mediated damage to schistosomula in vitro. Nature (Lond.), 252: 503-505.

Chasid, M.J., Dale, D.C., West, B.C. & Wolfe, S.M., 1975. The hyper-eosinophilic syndrome : Analysis of fourteen cases with review of the literature. Medicine: 54: 1-27.

Clegg, J.A. & Smithers, S.R., 1972. Effects of immune rhesus monkey serum on schistosomula of Schistosoma mansoni during cultivation in vitro. Int. J. Parasitol., 2: 79-98.

Coles, A.C., 1902. The blood in cases affected with filariasis and Bilharzia haematobia. Brit. med. J., 1: 1137-1138.

Colley, D.G., 1972. Schistosoma mansoni: eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. Exp. Parasitol., 32: 520-526.

Colley, D.G., 1973. Eosinophils and immune mechanism. I. Eosinophil stimulation promoter (ESP): a lymphokine induced by specific antigen or phytohemaggiutinin. J. Immunol., 110: 1419-1423.

Day, R.P., 1970. Eosinophil cell separation from human peripheral blood. Immunology, 18: 955-959.

Dean, D.A., Wistar, R. & Murrell, K.D., 1974. Combined in vitro effects of rat antibody and neutrophilic leukocytes on schistosomula of Schistosoma mansoni. Amer. J. trop. Med. Hyg., 23: 420-428.

DeWitt, W.B., 1953. Influence of Schistosoma mansoni infections on the eosinophil level of adrenalectomized mice. Exp. Parasitol., 2: 358-365.

Fine, D.P., Buchanan, R.D. & Colley, D.G., 1973. Schistosoma mansoni infection in mice depleted of thymus dependent lymphocytes. I. Eosinophilia and immunologic responses to a schistosomal egg preparation. Amer. J. Pathol., 71: 193-206.

Gleich, G.J. & Loegering, D.L., 1973. Selective stimulation and purification of eosinophils and neutrophils from guinea pig peritoneal fluids. J. Lab. clin. Med., 82: 522-528.

Gleich, G.J., Loegering, D.A. & Olson, G.M., 1975. Reactivity of rabbit antiserum to guinea pig eosinophils. J. Immunol., 115: 950-954.

Goetzl, E.J., Wasserman, S.I. & Frank Austen, K., 1975. Eosinophil polymorphonuclear leukocyte function in immediate hypersensitivity. Arch. Pathol., 99: 1-4.

Greene, B.M. & Colley, D.G., 1974. Eosinophils and immune mechanisms. II. Partial characterization of the lymphokine eosinophil stimulation promoter. J. Immunol., 113: 910-917.

Greene, B.M. & Colley, D.G., 1975. Production of the lymphokine eosinophil stimulation promoter by murine T lymphocytes. Fed. Proc., 34: 995.

James, S.L. & Colley, D.G., 1976. Evidence for a functional role for eosinophils in a parasitic infection. Fed. Proc., 35: 439.

Kazura, J.W., Mahmoud, A.A.F., Karb, K.S. & Warren, K.S., 1975. The lymphokine «eosinophil stimulation promoter» and human schistosomiasis mansoni. J. infect. Dis., 132: 702-706.

Mahmoud, A.A.F., in press. Purification of human granulocytes and production of the corresponding monospecific antisera. In: Bloom B. & David, J., (Eds.), «In Vitro Methods in Cell-Mediated Immunity». Academic Press.

Mahmoud, A.A.F., Kellermeyer, R.M. & Warren, K.S., 1974a. Production of monospecific rabbit antihuman eosinophil serums and demonstration of a blocking phenomenon. N. Engl. J. Med., 290: 417-420.

Mahmoud, A.A.F., Kellermeyer, R.W. & Warren, K.S., 1974b. Monospecific antigranulocyte sera against human neutrophils, eosinophils, basophils and myeloblasts. Lancet, 2: 1163-1166.

Mahmoud, A.A.F., Warren, K.S. & Boros, D.L., 1973. Production of a rabbit antimouse eosinophil serum with no cross-reactivity to neutrophils. J. exp. Med., 137: 1526-1531.

Mahmoud, A.A.F., Warren, K.S. & Graham, R.C., Jr., 1975a. Antieosinophil serum and the kinetics of eosinophilia in schistosomiasis mansoni. J. exp. Med., 142: 560-574.

Mahmoud, A.A.F., Warren, K.S. & Peters, P.A., 1975b. A role for the eosinophil in acquired resistance to Schistosoma mansoni infection as determined by anti-eosinophil serum.   J. exp. Med., 142: 805-813.

McMatton, J.E., 1967. A study of some clinicopathological manifestations in Schistosoma

mansoni infections in Tanzania. Ann. trop. Med. Parasitol., 61: 302-309.

Pelley, R.P., Karp, R., Mahmoud, A.A.F. & Warren, K.S., (in press). Antigen dose response and specificity of production of the lymphokine, eosinophil stimulation promoter. J. infect. Dis.

Russell, A.E., 1902. Eosinophilia associated with bilharzia disease. Lancet, 2: 1540.

Sher, A., MacKenzie, P. & Smithers, S.R., 1974. Decreased recovery of invading parasites from the lungs as a parameter of acquired immunity to schistosomiasis in the laboratory mouse. J. infect. Dis., 130: 626-633.

Sher, A., Smithers, S.R. & MacKenzie, P., 1975. Passive transfer of acquired resistance to Schistosoma mansoni in laboratory mice.   Parasitology, 70: 347-357.

Smithers, S.R. & Terry, R.J., 1969. Immunity in schistosomiasis. Ann. N.Y. Acad. Sci., 160: 826-840.

Sorour, M.F., 1930. The significance of the polymorphonuclear eosinophil cell and the association of bilharziasis and tuberculosis. J. Egypt. med. Assoc., 13: 73-79.

Speirs, R.S., 1970. Function of leukocytes in inflammation and immunity. In: Gordon, A.S. (Ed.) «Regulation of Hematopoiesis», Vol. 2. Appleton-Century-Crofts, New York, pp. 995-1043.

Warren, K.S., Karp, R., Pelley, R.P. & Mahmoud, A.A.F., (in press). The «Eosinophil Stimulation Promoter» test in murine and human Trichinella spiralis infection. J. infect. Dis.

Zucker-Franklin, D., 1974. Eosinophil function and disorders. Adv. intern. Med., 19: 1-25.

SUPPRESSION OF T-LYMPHOCYTES IN CHRONIC BILHARZIASIS*

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It is well known that patients with bilharziasis respond to the infection immunologically. High titers of schistosomal antibodies (Capron et al., 1969 ; G. Higashi, unpublished results), high levels of immunoglobulins (Michaux, 1966 ; Antunes et al., 1971 ; Bassily et al., 1972 ; El-Raziky et al., 1974), immediate and delayed type hypersensitivity skin reactions (Kagan & Pellegrino, 1961 ; Warren et al., 1973) and schistosomal antigen-reactive lymphocytes (Ky et al., 1969 ; Badawy & Mahfouz, 1975) have all been described. However, little attention has been devoted to a critical assessment of the immune status in various stages of the infection particularly in patients exhibiting manifestations of chronic bilharzial disease. At these latter stages of the disease, the host immune system has no doubt been subjected to repeated antigenic challenges not only from schistosomes but from other infectious agents (Lehman et al., 1972) and from autologous tissue antigens (Ekladios et al., 1971 ; Bassily et al., 1973).

In studies of the clinico-pathological spectrum of bilharziasis, we have observed that patients with chronic hepato-splenic bilharziasis exhibit some apparent abnormalities of the cell mediated immune system with specific emphasis on T-lymphocytes.

Patients and Methods

Patients

Thirty-one patients were admitted to the surgical wards of Cairo University Hospitals with massive splenomegaly, hematemesis or ascites. All had known histories of active bilharziasis. Thirteen patients underwent splenectomy at which time a wedge biopsy was removed from the liver. Histological examination of the liver biopsies uniformly showed severe hepatic fibrosis consistent with long-standing bilharziasis. Several biopsies grossly showed a pattern of fibrosis diagnosed as Symmers' clay pipestem fibrosis.

Lymphocytes

Peripheral blood lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation (Boyum, 1968). After washing three times in Hanks' balanced salt

solution (HBSS) the lymphocytes were counted in Turk's solution and adjusted : to 4×10^6/ml in HBSS containing 15% foetal calf serum (FCS) (HBSS-FCS) for rosette assays ; and to 1×10^6/ml in RPMI 1640 supplemented with antibiotics and 20% human group AB serum for culture.

Spontaneous erythrocyte (E) rosette assays (T Cells)

Two types were evaluated (David et al., 1974). First, the «active» T-cell population (E_1) was enumerated by incubating 0.2 ml lymphocytes with 0.2 ml of a 0.5% suspension of washed sheep red blood cells (SRBC) at 37°C for 1 hr, then centrifugating at 200 g for 5 min. and gently resuspending. The suspension was placed in a hemocytometer and 200 lymphocytes were counted including the number of rosette forming lymphocytes. A positive lymphocyte rosette had three or more SRBC bound to the lymphocyte membrane. The second T-cell population or total erythrocyte population (E_2) was determined by mixing lymphocytes and SRBC, incubating at 37°C for 15 min., centrifugation, and a further incubation overnight at 4°C. The assays were then made as above, but each tube was kept at 4°C until resuspension and counting.

EAC rosette assay (B-Cells)

The complement-antibody-coated erythrocyte (EAC) assay was done according to the method of Luckasen et al. (1974). Briefly, SRBC were sensitized with a subagglutinating dilution of rabbit anti-SRBC hemolysin, washed, coated with 1/20 dilution of mouse complement, washed and suspended (0.5%) in HBSS-FCS. Equal volumes of lymphocytes and EAC were centrifuged at 200 g for 5 min., then incubated at 37°C for 30 min. The cells were then vigorously resuspended

and the number of rosettes determined as above.

A further population of lymphocytes, those with immunoglobulin-Fc receptors, was determined by a rosette assay utilizing antibody coated SRBC (EA). The results show no differences between any of the groups studied and will not be described further.

Spleens

Thirteen patients underwent splenectomy after initial studies of peripheral blood lymphocytes were done. The spleens ranged in weight from 800 g to 2500 g. A randomly selected portion of each spleen was removed and the cells released by mincing with forceps and scalpel blades. Lymphocyte-enriched suspensions were obtained by Ficoll-Hypaque gradient centrifugation and processed as described for the various rosette assays. Cell viability determined by eosin Y dye exclusion (Hanks & Wallace, 1958) was usually over 80%. Four to six randomly selected blocks of spleen were fixed in 10% buffered formalin and processed for histopathologic evaluation. Routinely, hematoxylin and eosin and Giemsa stains were used.

Eight patients were followed up to four weeks after splenectomy. Peripheral blood lymphocytes were assayed for T and B cell populations by the various rosette tests.

Lymphocyte culture

Lymphocytes from six patients and six healthy volunteers were cultured. Phytohemagglutinin (PHA-P, 10 μg/ml final concentration) induced lymphocyte transformation was evaluated using 5×10^5 lymphocytes in closed plastic culture tubes for 72 hr incubation at 37°C. The last 18 hr contained 1 μC 3H-methyl

thymidine (2 Ci/mmol sp. act.). The cells were processed by standard procedures and the radioisotope incorporation determined in a liquid scintillation counter.

Skin tests

Ten units purified protein derivative of tuberculin (PPD, Connaught Medical Labs., Toronto), 20 units streptokinase-streptodornase (SK-SD, Lederle Labs., Pearl River, New York) and 20 μg Schistosoma mansoni egg crude extract were injected in 0.1 ml volumes intradermally. Responses were evaluated at 20 min., and at 24 or 48 hr for immediate and delayed skin responses, respectively.

Results

Summary data on E_1, E_2 and EAC populations enumerated in chronic bilharziasis patients' blood are shown in Table 1 and compared with those from 17 healthy controls. Statistical analysis (Student's t test) resulted in highly significant ($p < .001$) differences between patients and healthy controls as regards both E_1 and E_2. No significant difference was found in the EAC data.

T- and B-cell populations of lymphocytes obtained from the 13 surgical

spleens were compared with data published by Visakorpi & Repo (1973) relative to apparently normal spleens (Table 2). The T-lymphocyte figures were from E_2 assays to allow comparison to the normal figures. Our data reveal a marked drop in EAC rosetting lymphocytes compared to the normals, while the number of E_2 rosettes were slightly higher than normal although not significant statistically. Histological examination of 10 spleens showed a marked depletion of lymphocytes in the T-cell dependent areas, around the central arterioles and along the arterial sheaths, in five cases. Some depletion was evident in a further three cases and no evident change was noted in two cases. Germinal centers were abundant and active in all 10 spleens. Abundant eosinophils and clusters of typical plasma cells were evident in the medullary cord regions. No schistosome eggs or granulomas were found in any section.

Eight of the 13 patients who underwent splenectomy were studied four weeks after surgery. Both E_1 and E_2 lymphocyte populations were increased compared to preoperative values (Table 3). However, only the increase in E_2 was statistically significant.

TABLE 1. Rosette-forming lymphocyte subpopulations in peripheral blood of chronic bilharziasis patients.

Group (n)	Rosette-forming lymphocytes		
	E_1 Mean % ± S.D.	E_2 Mean % ± S.D.	EAC Mean % ± S.D.
Healthy Controls .... (17)	23.8 ± 12.6	66.1 ± 6.8	27.7 ± 7.6
Chronic Bilharziasis ...... (31)	15.2 ± 9.0	47.0 ± 14.0	29.3 ± 9.0

S. D. = standard deviation

MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A
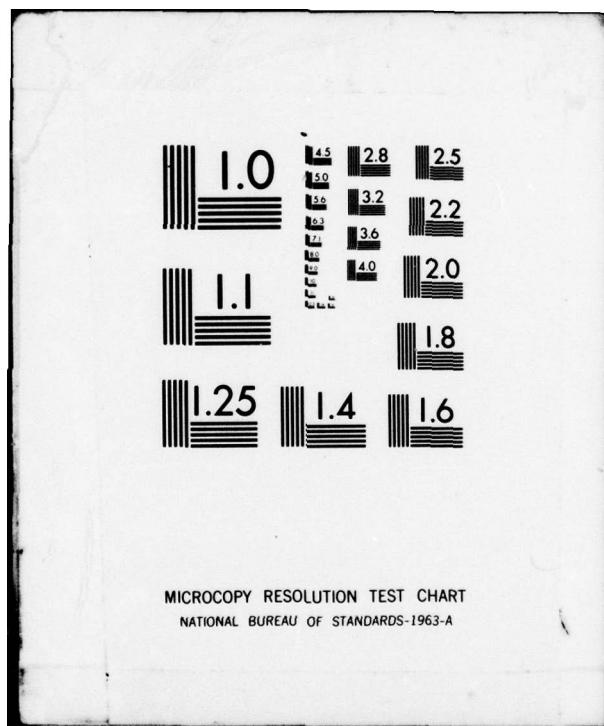
TABLE 2. Lymphocyte subpopulations in spleens from bilharziasis patients.

Group (n)	Rosette-forming cells	
	T lymphocytes Mean % ± S.D.	B lymphocytes Mean % ± S.D.
Normal . . . . (10)*	26.3 ± 8.9	58.0 ± 8.9
Bilharzial Splenomegaly . . (13)	35.4 ± 15.0	38.0 ± 18.5**

     * Figures from Visakorpi & Repo (1973).

     ** $P < 0.05$, Student's t test

TABLE 3. Effect of splenectomy on rosette-forming lymphocyte subpopulations in peripheral blood in eight bilharzial patients 4 weeks after surgery.

Time in rel. to Splenectomy	Rosete-forming lymphocytes		
	E_1 Mean % ± S.D.	E_2 Mean % ± S.D.	EAC Mean % ± S.D.
Preoperative	14.7 ± 7.0	36.6 ± 10.5	30.1 ± 12.0
Four weeks after	24.3 ± 13.5	54.0 ± 20.5*	32.5 ± 11.0

* $P < 0.05$, Student's t test

TABLE 4. Delayed hypersensitivity skin reactions in bilharzial patients.

Group (n)	No. of positive responders (≥ 5 mm)		
	PPD (10 U)	SK - SD (20 U)	S. mansoni Egg (20 µg)
Bilharziasis . . . . . (12)	0	2	0
Normal . . . . . . (12)	5	8	ND

U = units   PPD = purified protein derivative

ND = not done   SK-SD = streptokinase-streptodornase

Table 4 shows the results of delayed hypersensitivity skin tests to PPD, SK-SD and **Schistosoma mansoni** egg antigen. There were diminished responses to both PPD and SK-SD in most of the patients studied. Schistosome antigen was uniformly negative although a majority showed a typical immediate reaction manifested by a wheal and flare.

Preliminary studies on PHA-induced lymphocyte transformation in six patients showed marked depression of response in two patients (approximately 200 and 800 counts per minute). The other four had lymphocyte responses similar to or higher than those of six control cultures (4×10^3 cpm).

Discussion

The present study suggests that patients with chronic hepatosplenic bilharziasis manifest variable degrees of immunodepression involving the T-lymphocyte or cell mediated immune system. This depression was evidenced by diminished numbers of peripheral blood T-cells as detected by SRBC spontaneous rosettes and by depressed delayed skin reactions to microbial antigens. Preliminary data further suggest loss of lymphocyte responsiveness to PHA in culture in some patients. This type of secondary immunodeficiency is analogous to that found in other infectious diseases such as leprosy (Godal et al., 1971), malaria and trypanosomiasis (Talwar, 1974).

It should be noted that we have studied one stage of the disease spectrum in bilharziasis. Other earlier stages as well as the influence of the different species of human schistosomes may indeed manifest a competent immune status. Such comparisons are sorely needed to evaluate the relative immunological responses to schistosomes with respect to other concomitant infectious agents (e.g. salmonella, hepatitis A, B or C) and to autologous tissue antigens such as liver (Ekladios et al., 1971 ; Bassily et al., 1973) and colon (Kurata & Noda, 1965).

Based on the present preliminary studies, we might suggest that the spleen plays a significant role in the immunodepression observed in chronic bilharziasis. Although the T-cells in the spleen were somewhat higher than was described for normal spleens by Visakorpi & Repo (1973), the count approximates that of 36.5% recently reported for seven normal spleens by Habeshaw and Stuart (1974). However, the present histopathologic observations suggest a depletion of T-cells overall. It is possible that in such large spleens as those studied here, the total T-cell population may be closer to the absolute numbers found in normal spleens. Further work is required to assess quantitatively the absolute numbers of T and B lymphocytes in spleens from chronic bilharziasis. The relatively rapid increase in peripheral blood T-cells after splenectomy supports the hypothesis that the spleen plays a major role in our observations. Though the precise mechanisms for the effects of the spleen are unknown we can consider several possibilities. First, the enlarged spleen may be trapping the T-cells with subsequent destruction of sequestration. More attractive in contemporary immunobiology is that the spleen elaborates specific immunologic inhibitory factors and/or the spleen preferentially contains suppressor T-cells. Data in man are lacking although the recent immunologic literature is replete with studies in rodents showing that suppressor T-cells «home» to the spleen and can manifest profound immunodepression of various immunological reactions (Singal & Sinclair, 1975).

Extension of the present studies is needed to obtain data in support for any of the postulated mechanisms. Furthermore, the effect of splenectomy in bilharziasis patients must be immunologically evaluated on longterm follow-up to determine if functional immunocompetence returns, e.g. responses to skin tests and to

PHA-induced lymphocyte transformation. It also remains to determine how chronic bilharzial patients with secondary immunodeficiency respond to natural re-exposures to schistosome infections as well as to other infectious agents encountered in their environment.

LITERATURE CITED

Antunes, L.J., Reis, A.P., Pellegrino, J., Travares, C.A. & Katz, N., 1971. Immunoglobulins in human schistosomiasis mansoni. J. Parasitol. 57: 539.

Badawy, Y.H. & Mahfouz, K.A.H., 1975. Lymphoblastic transformation reaction test in bilharziasis. Med. J. Cairo Univ., 43: 45.

Bassily, S., Higashi, G.I., Farid, Z. & Williams, R.E., 1972. Serum Immunoglobulins in schistosomiasis mansoni. J. trop. Med. Hyg. 75: 73.

Bassily, S., Mohran, Y.Z., Said, M.I., Moussa, M., Iskander, F. & Mooro, H., 1973. Autoantibodies in schistosomal liver fibrosis. J. trop. Med. Hyg. 76: 153.

Boyum, A., 1968. Separation of leucocytes from blood and bone marrow. Scand. J. clin. Invest., 21 (suppl. 97), 109 pp.

Capron, A., Vernes, A., Biguet, J., Rose, F., Clay, A. & Adenis, L., 1966. Les précipitines sériques dans les Bilharzioses humaines et expérimentales à Schistosoma mansoni, S. haematobium et S. japonicum. Ann. Parasitol. 41: 123.

David, T.Y., Clements, P.J., Paulus, H.E., Peter, J.B., Levy, J. & Barnett, E.V., 1974. Human lymphocyte subpopulations. Effect of cortisone. J. clin. Inv. 53: 563.

Ekladios, E.M., Ageeb, M., Milad, M. & Khairy, M., 1971. Auto-antibodies in hepatic schistosomiasis. Kasr El Aini J. Surg. 12: 299.

El-Raziky, E.H., Shaker, Z.A., Mahmoud, A.H., Kamel, K., Abou El-Ezz, F.M. & Mousa, A.H., 1974. Immunoglobulins in schistosomiasis. Egypt. J. Bilharz. 1: 287.

Godal, T., Myklestad, B., Samuel, D.R. & Myrvang, B., 1971. Characterization of the cellular immune defect in lepromatous leprosy. Clin. Exp. Immunol. 9: 821.

Habeshaw, J.A. & Stuart, A.E., 1974. T. and B. cells in human spleen. Lancet, 1: 1164.

Hanks, J.H. & Wallace, J.H., 1958. Determination of cell viability. Proc. Soc. exp. Biol. Med. 98: 188

Kagan, I.G. & Pellegrino, J., 1961. A critical review of immunological methods for the diagnosis of bilharziasis. Bull. Wld Hlth Org. 25: 611.

Kurata, M. & Noda, R., 1965. Detection of circulating «autoantibodies» in experimental schistosomiasis japonica. Kurume Med. J. 12: 1.

Ky, M.T., Halpern, B., Amache, N., LaRoche, C. & LaRoche, L., 1969. Lymphocyte culture in the immunological diagnosis of human bilharziosis. Acta Allergol. 24: 76.

Lehman, J.S. Jr., Higashi, G.I., Bassily, S. & Farid, Z., 1972. Rheumatoid factors in Salmonella and Schistosoma infections. Tr. r. Soc. trop. Med. Hyg. 66: 125.

Luckasen, J.R., Sabad, A., Gajl-Peczalska, K.J. & Kersey, J.H., 1974. Lymphocytes bearing complement receptors, surface immunoglobulins and sheep erythrocyte receptors in primary immunodeficiency diseases. Clin. exp. Immunol. 16: 535.

Michaux, J.L., 1966. Les immunoglobulines des Bantous à l'état normal et pathologiques. Ann. Soc. Belg. Med. trop. 46: 483.

Singhal, S.K. & Sinclair, N.R. St. C. (Eds.), 1975. Suppressor cells in immunity, 210 pp., The University of Western Ontario, Ontario, Canada.

Talwar, G.P., 1974. Immunodeficiency secondary to other diseases. Progress in Immunology II, 5: 366.

Visakorpi, R. & Repo, H., 1973. T-lymphocytes in human spleen. Lancet, 2: 740.

Warren, K.S., Kellermeyer, R.W., Jordan, P., Littell, A.S., Cook, J.A. & Kagan, I.G., 1973. Immunologic diagnosis of schistosomiasis. I. A controlled study of intradermal (immediate and delayed) and serologic tests in St. Lucians infected with Schistosoma mansoni and in uninfected St. Vincentians. Amer. J. trop. Med. Hyg. 23: 189.

IMPAIRED IMMUNOLOGIC REACTIVITY IN PATIENTS WITH URINARY BLADDER CANCER ASSOCIATED WITH BILHARZIASIS

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There is abundant epidemiological evidence indicating the association between *Schistosoma haematobium* and urinary bladder carcinoma in Egypt (El-Sebai, 1961 ; Abdel Tawab, 1966). However, virtually nothing is known of the precise mechanism by which *S. haematobium* infection ultimately leads to carcinoma. Furthermore it is not known whether immunological control mechanisms, such as immune surveillance, play a significant role in the development and progression of bilharzial associated squamous cell carcinoma of the urinary bladder.

As a preliminary step regarding in depth studies of urinary bladder carcinoma associated with bilharziasis, we wish to present results on an initial study on the evaluation of the immune status of such patients.

Thymus-derived lymphocytes (T-lymphocytes) and bone marrow derived lymphocytes (B-lymphocytes) in the peripheral blood were enumerated by the various erythrocyte rosette assays. Furthermore, delayed hypersensitivity-type skin tests with common microbial antigens were performed.

Material and Methods

Patients and controls

Twelve healthy volunteers served as controls. They were of comparable age and sex distribution to the 20 bladder cancer patients studied (Table 1). Clinical staging of patients was done according to the Wallace classification. Tumor grading was done after histopathological and

TABLE 1. **Data on persons investigated**

I. Controls (12)	Age: 29 - 50 yrs; 10 M/2F
II. Patients (20)	Age: 27 - 53 yrs; 16 M/4F
Squamous cell carcinoma (13):	
- grade I	6
- grade II	4
- grade III	3
Transitional cell carcinoma (4):	
- grade I	1
- grade II & III	2
- grade IV	1
Adenocarcinoma	1
Unclassifed	2

exfoliative cytological examination. None of the patients had received any type of anticancer treatment. All patients had a known history of *S. haematobium* infection and had stopped antibilharzial treatment for at least one month before the start of the study. Histories of blood transfusion or anti-inflammatory or immunosuppressive drug administration to patients or controls were negative.

Isolation of peripheral blood lymphocytes

Blood lymphocytes were isolated according to Boyum (1968). Heparinized blood was diluted with three parts of phosphate buffered saline (PBS). 20 ml of diluted blood was layered on top of 8 ml of Ficoll-isopaque gradient, spun at $400 \times g$ for 30 min at $17^\circ C$ and the cell rich interface was collected and washed three times in PBS. Lymphocytes cell suspension was adjusted to 4×10^6 cells/ml in Hanks' balanced salt solution containing 15% foetal calf serum.

Erythrocyte (E_1 and E_2) rosette assay (T-cells)

E_1 and E_2 rosette assays were done according to David et al. (1974) after slight modification. 0.2 ml of washed lymphocytes cell suspension was mixed with an equal volume of 0.5% suspension of washed sheep erythrocytes (SRBC), incubated at $37^\circ C$ for 1 hr, spun at $200 \times g$ for 5 min., gently mixed and then placed in a haemocytometer for counting rosette forming cells. A rosette was defined as a lymphocyte with three or more SRBC attached to its surface. The above assay will give the percentage of E_1 rosettes. The total T-cell population (E_2) was counted after incubating lymphocytes with SRBC at $37^\circ C$ for 15 min., centrifuged and kept at $4^\circ C$ overnight. Rosette counts were made as described.

EA and EAC rosette assay (B-cells)

Antibody-coated (EA) and complement antibody-coated (EAC) erythrocyte rosette assay was done according to Luckasen et al. (1974) after certain modifications. To 5% washed SRBC was added an equal volume of rabbit anti-sheep antibody diluted below its haemagglutinating dilution (about 1:5000) ; the mixture, incubated at $37^\circ C$ for 30 min. was washed in PBS. The coated SRBC (EA) were resuspended to 5% and incubated with a 1:20 dilution of fresh frozen mouse complement at $37^\circ C$ for 30 min. They were then washed and the final concentration was adjusted to 1% in order to obtain EAC cells. Equal volumes of lymphocytes and either EA or EAC were centrifuged at $200 \times g$ for 5 min., then incubated at $37^\circ C$ for 30 min. The number of rosette forming lymphocytes was counted in a haemocytometer after vigorous shaking using a Vortex mixer.

Peripheral blood lymphocyte count

A total leucocytic count was done for the blood sample used in the rosette assay using a haemocytometer. Leishman stained smears were used for differential leucocyte counting.

Delayed skin hypersensitivity reaction to common microbial antigen

Ten units of purified protein derivative (PPD) of tuberculin (Connaught Medical Labs., Toronto) and SK-SD (10 units streptokinase and 2.5 units streptodonase ; Lederle Labs., Pearl River, N.Y.) were injected intradermally. Both antigens were injected in 0.1 ml volumes in the volar face of the forearm. Reaction to antigens at 48 hr was measured. Erythema and induration greater than 5 mm in diameter was considered positive.

Results

Counts of rosette forming lymphocytes (Tables 2, 3)

The mean percentage of E_2 rosette forming lymphocytes (T-lymphocytes) and of null lymphocytes of the bladder cancer patients were significantly lower than those of the healthy controls (48.4 and 64.8% respectively). However, EA and EAC rosette forming lymphocytes counts (B-lymphocytes) of the bladder cancer patients were higher than those of the healthy controls although that did not reach statistical significance (Table 2). The decrease in the counts of E_2-rosettes in patients with late stages of bladder cancer (stages III & IV) was much more apparent than in those with early stages (stages I & II) as shown in Table 4.

TABLE 2. Rosette forming lymphocytes in peripheral blood of patients with urinary bladder carcinoma.

	Healthy Controls Mean %± S.E.M.	Bladder Carcinoma Mean %± S.E.M.
No.	12	20
Mean age	35.9 ± 2.1	40.9 ± 1.5
E_1	21.8 ± 3.2	18.4 ± 3.0
E_2	64.8 ± 2.1	48.4 ± 3.2*
EA	20.4 ± 4.5	25.7 ± 4.2
EAC	25.3 ± 3.4	28.4 ± 2.7
Null	11.8 ± 4.0	24.0 ± 4.6**

S.E.M = Standard error of mean
* P   0.001   - Student's t test
** P =0.05   - Student's t test

TABLE 3. Absolute rosette forming lymphocyte counts in peripheral blood of patients with urinary bladder carcinoma.

Counts	Absolute lymphocyte counts/cu. mm	
	Healthy Controls Means ± S.E.M.	Bladder Carcinoma Means ± S.E.M.
Total lymphocytes	1768 + 105.7	1148 + 102.0*
E_1	400.6 ± 62.8	237.3 ± 69.0**
E_2	1148 ± 82.0	564 ± 70.7*
EA	342.8 ± 72.6	284.6 ± 56.0
EAC	444 ± 65.5	333 ± 47.0
Null	208.5 ± 72.0	286.9 ± 55.4

* P   0.001   - Student's t test
** P =   0.05   - Student's t test

Absolute counts of rosette forming lymphocytes (Table 3)

Total lymphocyte counts of cancer patients were significantly lower compared to healthy controls. It is clear that the total T-cell counts (E_1 and E_2-rosettes) gave significantly lower results in cancer patients while EA and EAC-rosettes and null cell counts did not show a significant change compared to healthy controls.

Skin tests (Table 5)

All healthy controls had positive skin reactions (above 5 mm) to PPD and SK-SD except one case who was negative to PPD test. Fourteen out of 20 patients had positive skin reactions to PPD and SK-SD. The difference between the positive skin reaction of patients and that of healthy controls was not significant. The mean diameter of induration was significantly lower in bladder cancer patients compared to healthy controls.

TABLE 4. Comparison of rosette forming lymphocytes in the peripheral blood of healthy controls and patients with different stages of bladder carcinoma.

Condition	Number	E_2 - rosette forming lymphocytes (%) ± S.E.M.	Statistical significance (Student's t test)
Healthy Controls	12	64.8 ± 2.1	—
Carcinoma Stage I & II	13	53.2 ± 2.7	P = 0.01
Carcinoma Stage III & IV	7	39.4 ± 6.7	P 0.001

TABLE 5. Delayed hypersensitivity skin tests in patients with urinary bladder carcinoma and healthy controls.

Antigen	Healthy Controls			Bladder Carcinoma		
	No.	Response 5 mm	Mean Diameter mm (± SEM)	No.	Response 5 mm	Mean Diameter mm (± SEM)
PPD	12	11	19 + 3.0	20	14	11 ± 0.9*
SK - SD	12	12	20 ± 3.7	20	14	12 ± 1.0*

* P 0.05   - Student's t test.

PPD   =   Purified protein derivative of tuberculin.

SK-SD =   Streptokinase-streptodornase

Discussion

This study provides evidence of an impairment in the cellular immune response of patients with bladder cancer, represented by the low numbers of circulating T-lymphocytes, lymphopenia and the lower intradermal responses to common microbial antigens.

It has been suggested that the rate of tumor growth in cancer patients might depend on the balance between the T and B-lymphocyte populations (Good, 1970). In our study, T-cells were significantly decreased while B-cells were increased, although insignificantly, in the peripheral blood of patients with bladder cancer. In addition a third cell population, null cells, appeared in their circulation. The increase in the percentage of null cells in the peripheral blood of cancer patients might be a real phenomenon. Null cells might be explained as being immature forms of lymphocytes (T and B) which lack membrane receptors active in rosette formation. However, the latter suggestion might not be accepted because there was little difference between the absolute number of null cells of bladder cancer patients and that of healthy controls (Table 3). Accordingly lymphopenia was mainly due to the decrease in T-cell number (E_2-rosettes) and partially, due to B cells (EAC-rosettes) (Table 3).

The significant decrease in E_2-rosette forming lymphocytes (T-cells) in bladder cancer patients could be shown in all of the different stages of the disease (Table 4). However the decrease was of higher significance in patients with advanced cancer. Catalona et al. (1974) showed a significant decrease in T-cell population in the peripheral blood of patients bearing an advanced bladder cancer but the decrease was not significant in patients with early bladder cancer. Similar results to those of Catalona were shown in lung cancer (Brugarolas et al., 1973), gastric cancer (Orita et al., 1974) and other cancers (Tachibana & Ishikawa, 1973 ; Wybran & Fudenberg, 1973). The difference between our results and those of Catalona et al. (1974), might be due to the fact that two different groups of patients were studied. Our patients suffered mainly from the squamous cell carcinoma type (Table 1) which is associated with bilharziasis, while those of Catalona et al., most probably had transitional cell carcinomas. It might be concluded that the bilharzial bladder carcinoma of Egypt, selected according to the usual distribution of age, sex and histopathological types of that cancer, as studied by El Boulkainy et al. (1972) might constitute a special immunological entity, or might have a different etiology from that of nonbilharzial bladder cancer. Whether the early immune deficiency is a prerequisite for the induction of bilharzial bladder cancer or is a resultant of it, is open for discussion and further studies. However, the concomitant impaired cell mediated immune response in bilharzial bladder cancer ought not to be interpreted as being based on the concomitant bilharzial infection. In another paper read at this conference (Nooman et al., 1978) it was shown that cell mediated immune responses of patients with pure *S. haematobium* infection, tested by *in vitro* methods, were intact. However, in studies also presented to this conference, Ekladios et al. (1978) showed that the percentage of E_2-rosette forming lymphocytes (T-cells) of the peripheral blood of patients with chronic hepatosplenic bilharziasis was significantly lower than that of healthy controls. Single urine and stool examinations for bilharzial eggs were negative for all of our group of cancer patients, although Cheever et al. (1975), in a separate study on bladder cancer patients, found that a variable number of eggs were still being excreted when examining the 24 hr urine samples. It might be more relevant to study the potential effect of bilharziasis on the immune state of patients with recurrent bilharzial cystitis, a disease which predisposes for bladder cancer in Egypt.

Our preliminary results draw our attention to the significant depression in the immune status of bilharzial bladder cancer patients which suggests that immunotherapy would be useful especially in the early forms of the disease. Such treatment should be chosen after careful and thorough evaluation of the immune state of such patients, aiming at clearer clinical staging of bilharzial bladder cancer. Then the most efficacious mode of therapy (immunotherapy, chemotherapy, radiotherapy, surgery) may be more rationally applied.

LITERATURE CITED

Abdel Tawab, G.A., 1966. Studies on the etiology of the bilharzial carcinoma of the urinary bladder. Int. J. of Cancer, 1: 377.

Boyum, A., 1968. Separation of leukocytes from blood and bone marrow. Scand. J. clin. Invest., 21 (Suppl. 97): 109.

Brugarolas, A., Han, T., Takita, H. & Minowada, I., 1973. Immunologic assays in lung cancer. Skin tests, lymphocyte blastogenesis, and rosette forming cell counts. N.Y. State J. Med., 73: 474.

Catalona, W.J., Potvin C. & Chretein, P.B., 1974. T-lymphocytes in bladder and prostatic cancer patients. J. Urology, 112: 378.

Cheever, A.W., Torky, A.H. & Sherbiney, M., 1975. The relation of worm burden to passage of Schistosoma haematobium eggs in the urine of infected patients. Amer. J. trop. Med. Hyg. 24: 284.

David, T.Y., Clements, P.J., Paulus, H.E., Peter, J.B., Levy, J. & Barnett, E.V. 1974. Human lymphocyte subpopulations. Effect of cortisone, J. clin. Inv. 53: 563.

Ekladios, E., Higashi, G.I., Ageeb, M. & El Ghorab, Nemat M., 1975. Suppression of T-lymphocytes in chronic bilharziasis. Proc. internat. Conf. Schist., Cairo.

El-Boulkainy, M.N., Ghoneim, M.A. & Mansour, M.A., 1972. Carcinoma of the bilharzial bladder in Egypt. Brit. J. Urol. 44: 561.

El-Sebai, I., 1961. Cancer of the bladder in Egypt. Kasr El Aini J. Surg. 2: 183.

Good, R.A., 1970. Critique of surveillance hypothesis. In immune surveillance. Ed.: R.T. Smith & M. Landy. N.Y. Ac. Press. pp. 439.

Luckasen, J.R., Sabad, A. Grajl-Peczalska, K.J. & Kersey, J.H., 1974. Lymphocytes bearing complement receptors, surface immunoglobulins and sheep erythrocyte receptors in primary immunodeficiency diseases. Clin. Exp. Immunol. 16: 535.

Nooman, Z.M., Oreiby, A.M. & Higashi, G.I., 1978. Delayed hypersensitivity in patients with bilharzial hepatic fibrosis. Proc. intern. Conf. Schisto., Cairo.

Orita, K., Miwa, H., Ogawa, K., Suzuki, K., Sakagami, K., Konaga, E., Kokumai, Y. & Tanaka, S., 1974. Reduction in immunological surveillance level in cancer patients. Gann Monograph, 16: 53.

Tachibana, T. & Ishikawa, M., 1973. A new micromethod for quantitation of human T- and B-lymphocytes. Jap. J. exp. Med. 43: 227.

Wybran, J. & Fudenberg, H.H., 1973. Thymus-derived rosette forming cells in various human disease states: cancer, lymphoma, bacterial and viral infections and other diseases. J. clin. Invest. 52: 1026.

SYNTHESIS OF MACROMOLECULES BY THE EPITHELIAL SURFACES OF SCHISTOSOMA MANSONI, WITH PARTICULAR REFERENCE TO THE FATE OF SECRETORY VESICLES IN THE WORM TEGUMENT

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The results presented here are part of an investigation into the cell biology of *Schistosoma mansoni* in relation to the immune responses of its mammalian host. The work falls into two parts : a consideration of the capacity of the different epithelial surfaces of the worm to produce antigenic macromolecules, and a consideration of the manner in which processes occurring in one of these surfaces, the tegument, might be related to possible mechanism for evasion of the host immune response.

Synthesis of secretory macromolecules

The epithelial surfaces which we have investigated in this study are the cells of the intestine, the protonephridial system, and the tegument with associated cell bodies. The epithelia of the male and female reproductive system have not been included because of their complexity of structure and function. It was first necessary to estimate the relative sizes of the different metabolic pools involved in the secretory processes. We did this by calculating the proportion of total area occupied by the different epithelia in transverse sections of male and female worms. For male worms the results were as follows : Parenchyma 56%, Dorsal musculature 22.5%, Intestinal epithelium 4%, Intestinal lumen 3%, Ventral musculature 2.5%, Dorsal tegument and associated tegumental cells 8% and Ventral tegu-

ment 2.5%. The protonephridial system comprises less than 1% of total worm tissues.

The technique selected to investigate the synthetic and secretory ability of the different epithelial surfaces was the incorporation of isotopically labelled precursors into macromolecules, followed by autoradiography. Two isotopes were selected ^{3}H leucine as an index of protein synthesis and ^{3}H glucosamine as an index of polysaccharide synthesis. Details of technique used are as follows : Worms were incubated in balanced salt solution containing the isotopically labelled compound for a pulse period of 30 min. duration. A sample of worms was then washed and fixed. The remainder were thoroughly washed with double changes of medium and transferred to fresh culture medium containing unlabelled leucine or glucosamine for a chase incubation of varying duration. At the end of the chase period a sample of worms was washed, fixed, dehydrated and embedded in Araldite. Sections were cut for light and electron microscope autoradiography. They were then coated with a monolayer of photographic emulsion and left in the dark for varying periods of time to permit adequate detection of isotopic disintegrations. The photographic emulsion was then developed and fixed. The presence of silver grains over the light and electron microscope sections gives an in-

dication of the position of macromolecules into which leucine or glucosamine have been incorporated. The relative density of grains is an index of the amount of protein or polysaccharide in that tissue as compared with other tissues in the worm. The change in the density of grains over a particular tissue with time indicates the rate at which protein or polysaccharide moves from one tissue to another or is exported from the worm. From these results it is possible to calculate the half life of a secretory protein in the cell where it was synthesised or in the organ into which it was secreted.

Protein synthesis

The results of autoradiography indicate that the tegument cell bodies, the intestinal epithelial cells and the cells of the protonephridial system are all capable of synthesising leucine-containing protein. The distribution of grains immediately following the pulse period indicates that the tegument cells synthesise about twice as much secretory protein as the intestinal cells. The half life of the protein in the tegument cell bodies is 2-3 hr. The half life of protein in the tegument cell bodies and tegument treated as a single compartment is approximately 4 hr. The half life of protein in the intestinal epithelial cells is 2.5-3.5 hr and that in the intestine plus intestinal lumen is 3-4.5 hr. This suggests that the intestinal contents are being replaced very rapidly, a contention supported by the work of Lawrence (1973).

Polysaccharide synthesis

Both tegument cell bodies and intestinal epithelial cells are capable of synthesising glucosamine-containing polysaccharide. Relative grain counts indicate that the intestine synthesises and secretes 90% or more of the polysaccharide exported by the worm. The half life of the

polysaccharide in the intestinal cells is approximately 3 hr; its half life in the lumen of the intestine is greater than 24 hr. The polysaccharide, once secreted, appears not to disperse and we believe that it comprises the prominent glycocalyx of the intestinal epithelial cells (Wilson & Barnes, 1974a). There is thus a considerable disparity between the turnover of proteins and polysaccharides secreted into the intestinal lumen.

Secretory processes in the tegument

There are two major secretory vesicles produced in the subtegumental cell bodies and found in the tegument ground substance. These are :

a) Discoid granule. This inclusion is carbohydrate-positive but we have no real idea of either its fate or its function.

b) Multilaminate vesicle. Histochemistry at the electron microscope level indicates the presence of quantities of lipid and also traces of carbohydrate in this vesicle. It should be noted that the bounding membrane of the multilaminate vesicle has properties different from the laminate contents.

Fate of the multilaminate vesicle

The use of rapid fixatives for electron microscopy such as Karnovsky's or Mollenhauer's fixatives has enabled us to observe the fate of multilaminate vesicles in the schistosome tegument. The structure of the tegument is in fact more complicated than is indicated in the descriptions of such workers as Morris & Threadgold (1968) and Smith et al. (1969). Minor side channels run out into the cytoplasm from the base of the major tegumental pits. These side channels may be transient structures and the multilaminate vesicles appear to fuse with their tips. The laminate contents of the ve-

sicles are then released into the side channels and main channels of the tegument. Hockley & McLaren (1973) have described the multilaminate nature of the surface plasma membrane of the schistosome tegument. This consists of seven and sometimes as many a nine or eleven separate lamellae. We have suggested (Wilson & Barnes, 1974b) that this multilaminate appearance is produced by the laminate secretion of the multilaminate vesicles lying on top or outside the normal plasma membrane which bounds the tegument. We described these laminate secretions as a membranocalyx by analogy with the glycocalyx which coats the surface of many epithelial cells.

Since the process of vesicle incorporation into the tegument surface is continuous it follows that the membranocalyx must be turning over. We have attempted to measure the rate of turnover of the membranocalyx by attaching to it extrinsic markers or labels and then following the rate at which these markers are released into the incubation medium. One such marker we have used is a plant lectin Concanavalin A. When worms are incubated in vitro in a medium containing Concanavalin A, the process of vesicle fusion is halted, and large numbers of vesicles are trapped in the act of releasing their secretions. We hope as a result of this experiment to be able to measure the rate at which vesicles are incorporated into the tegument surface. The ability of Concanavalin A to bind to the membranocalyx of the tegument implies the presence of polysaccharides in the membranocalyx, a finding which agrees with histochemical observations at the electromicroscope level.

Using the molecule cationised ferritin as a label we were able to successfully measure the rate of membranocalyx turnover. Worms were incubated for a period 30 min. in medium containing cationised ferritin. They were then either fixed, or post incubated in culture medium alone for varying periods of time up to 4 hr. After 30 min. incubation in cationised ferritin, the membranocalyx on the surface and in the side channels was heavily labelled with cationised ferritin. Two hours later the side channels were partially free of cationised ferritin presumably as a result of the incorporation of fresh membranocalyx material into the channels. After 4 hr chase incubation the bulk of ferritin-labelled membranocalyx had been released into the incubation medium and most side channels were completely cleared. The membranocalyx coating the tegumental spines still possessed some ferritin label, suggesting a conveyor belt-like movement of membranocalyx from the side channels onto the tegument surface, up the sides of spines and out into the incubation medium. Thus under the conditions of these experiments it would appear that the membranocalyx can be completely replaced in less than 4 hr.

What is the relevance of this process of membranocalyx turnover to mechanisms by which the worm might evade the immune response of its host ? At least two such mechanisms have been proposed by other workers, host antigen disguise and molecular mimicry. To these we would like to add a third possible mechanism, the rapid turnover of a membranocalyx barrier on the external surface of the tegument. This barrier might operate in many possible ways, e.g. by interfering with complement fixation, by inhibiting macrophages and lymphocytes or by interacting with humoral antibodies directed against the worms surface. It is, therefore, of vital importance to discover more about the composition and function of the membranocalyx.

LITERATURE CITED

Hockley, D.J. & McLaren, D.J., 1973. Schistosoma mansoni: changes in the outer membrane of the tegument during development from cercaria to adult worm. Internat. J. Parasit. 3: 13-25.

Lawrence, J.D., 1973. The ingestion of red blood cells by Schistosoma mansoni. J. Parasit. 59: 60-63.

Morris, G.P. & Threadgold, L.T., 1968. Ultrastructure of the tegument of adult Schistosoma mansoni. J. Parasit. 54: 15-27.

Smith, J.H., Reynolds, E.S. & von Lichtenberg, F., 1969. The integument of Schistosoma mansoni. Amer. J. trop. Med. Hyg., 18: 28-49.

Wilson, R.A. & Barnes, P.E., 1974a. The tegument of Schistosoma mansoni : observations on the formation, structure and composition of cytoplasmic inclusions in relation to tegument function. Parasitology 68: 239-258.

Wilson, R.A. & Barnes, P.E., 1974b. An in vitro investigation of dynamic processes occurring in the schistosome tegument, using compounds known to disrupt secretory processes. Parasitology 68: 259-270.

IMMUNOPATHOLOGICAL STUDY OF GLOMERULONEPHRITIS ASSOCIATED WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION

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As clinicians we have long been intrigued by the frequent association between schistosomiasis and nephrotic syndrome : schistosomiasis affected 57% of 140 nephrotic patients observed over the period 1959-1961 in the Ain Shams University Hospital and 47% of nephrotics seen in the Assiut University Hospital renal clinic were harbouring *Schistosoma haematobium*.

As glomerulonephritis is now considered to be mostly of immunological origin, either due to immune complex deposition or to glomerular basement membrane injury by antibodies, we planned, in 1971, to study the possible immunological role of schistosomiasis in inducing glomerular damage.

Only males between the ages of 10-50 years were included. They all fulfilled the parameters diagnostic of the nephrotic syndrome : heavy proteinuria, hypo-albuminaemia and a glomerular permeability index of more than 1×10^{-3}. Care was taken to exclude subjects with any of the known nephritis inducing antigens or with streptococcal infection, with malaria and with infections of the urinary tract. The urine was examined for the degree of proteinuria, schistosome ova, evidence of active glomerulonephritis, rate of cellular excretion and for casts. Immunoglobulin (Ig) excretion was measured, sigmoidoscopy was performed and a rectal

snip was obtained so as to search for embedded ova. The blood picture was taken and a detailed examination was made of the biochemical and immunological pattern of the serum, i.e. for serum proteins, serum cholesterol, blood urea, serum creatinine, serum immunoglobulins, the rheumatoid (Rh) factor, circulating worm and egg antigens and antibodies. The serum complement profile was determined.

Kidney biopsy specimens were examined for histopathological changes, cultured for bacteria and submitted to immunofluorescent studies. Kidney specimens were immediately snap frozen in liquid nitrogen and sections of 4-6 μm thickness were cut in a cryostat. Dried preparations were washed, fixed and stained with fluorescein isothiocyanate-conjugated monospecific antisera, rewashed, mounted and examined for fluorescent deposits using an Immunopan Fluorescent Microscope (Reichert). Conjugated antisera used were anti-IgG, IgM, IgA and IgE. Antisera to the complement components C3, C4 and C3PA (proactivator), to fibrinogen and albumin were also used. An antischistosomal fluorescein-conjugate was prepared by labeling the IgG of human sera containing a high level of anti-worm antibodies. All sera were negative for the Rh factor. Pooled sera were absorbed by human Rheumatoid negative group O red cells and by foetal

kidney powder. Specificity for anti-*Schistosoma* worm antibody was tested by getting a positive fluorescence on application of the conjugated globulin to a slide with frozen sections of unfixed adult *S. haematobium* worms.

Results so far Obtained

From 300 nephrotics, 100 fulfilled the criteria of *Schistosoma* associated glomerulonephritis. These patients presented :

1. Definite positive evidence of schistosomal lesion : ova in urine or rectal snip.

2. A negative response to various known antigens ; low antistreptolysin O titre (ASOT), no systemic bacterial infection ; they were negative for malaria, hepatitis B antigen and pyelonephritis ; normal intravenous pyelogram. Repeated negative urine culture and negative biopsy specimen culture.

3. They also presented urinary evidence of active glomerulonephritis : increase of rate of renal epithelial cellular excretion/hr, and casts.

Clinical characteristics

35% of the patients were in their second or third decade of life (i.e. from 15 to 25 years old). The history of schistosomiasis was short or transient and the symptoms were almost always mild. In 20 cases oedema was acute at the onset and preceded by 5-7 days of fever. In the remainder it was insidious.

Ascites, preceding oedema, was found in 38 patients. Forty patients mentioned a history of intermittent attacks of mild fever, skin rash and pain in the joints. Seventeen were actually suffering from rheumatoid-like arthritis, which disappeared within days.

Blood pressure was normal in 87 and raised in 13. Thirty-eight patients were asthmatic. Seventy cases showed various degrees of hepatic involvement : mild or moderate, a soft or firm enlargement. Actual shrinking was noted in 13 cases. No jaundice. Sigmoidoscopy disclosed the presence of polyps with *S. haematobium* ova in 10 cases. Rectal snips were positive for ova in 78 patients, of whom 40 had no ova in the urine.

Blood chemistry

Serum proteins ranged from 3 to 8 g/100 ml. Albumin was always less than 3 g/100 ml, ranging from 0.5 to 2.5 g/100 ml. Alpha 2 globulins were only raised in the absence of severe liver involvement. Serum cholesterol was more than 250 mg/100 ml in 35 cases and normal in the rest. Blood urea was normal in 77 cases, 60-70 mg/100 ml in 19 cases, and above 90 mg/100 ml in four. Ten cases had a creatinine clearance rate of 60-90 ml. Serum IgG 800-1800, serum IgM 100-220, urine IgG 400-600, urine IgM 10-22 mg/100 ml and the clearance rate of Ig/albumin was more than 0.2.

The titre of the rheumatoid factor was elevated in active nephritis, in liver fibrosis and in the presence of circulating antibodies.

Circulating worm antigens were detected by immunoelectrophoresis in 25 out of 50 sera studied.

Morphological features

Histologic examination disclosed a rather uniform pattern of glomerular enlargement, mesangial widening, capillary wall thickening and hypercellularity. However within this framework three main types could be differentiated according to the degree of mesangial matrix swelling, cellular proliferation and capillary wall thickening :

Type I, with increased density and fuzziness of the glomerular stalk and a mild increase in the mesangial cells.

Type II, which was the most common. Here the glomerulus was enlarged, with increased density and widening of the mesangial stalk ; there occurred massive cellular proliferation with obliteration of the tuft. The capsule and peripheral capillaries were irregularly thickened by a PAS + material. In this group two sub-groups (a & b) could be differentiated : group IIa, where hypercellularity was more marked than capillary wall thickening, and group IIb, in which marked thickening of the capillary wall dominated the picture and tuft lobulation was exaggerated.

Type III showed marked lobulation of the tuft, epithelial proliferation and a laminated appearance of the capillary wall. Subendothelial Masson green deposits were evident. Periglomerular cellular infiltration was found in all types.

Immunofluorescence

Immunoglobulins G, M and A were tested for in 80 specimens :

a) IgG deposits were found in 73 specimens. Most commonly all structures did stain, whereas in 12 cases only the glomerulus was stained. Fluorescence was usually in the form of conglomerated fine granular deposits in the mesangium and along the vessel wall. There was some variation in the size of the granules which varied inversely to the severity of the lesion. The intensity of fluorescence was more marked in cases with circulating antibodies. IgG was mostly found in types II and III.

b) IgM was deposited in 62 specimens. Deposits were localized in the glomerulus in 16 cases. Deposits were coarsely granular and evenly distributed. They were more common and distinct in Type I, especially with recent onset. Patients usually had highly or moderately selective proteinuria. It was less distinct and less frequent in Type II while it was almost absent or only focal in Type III. It was absent from the glomerulus and only found in vessels and tubules in cases which also showed a negative stain for IgG.

c) IgA was deposited in 23 biopsy specimens. It was mostly found in Type IIb. Serum IgA was normal but IgG was always higher than 2500 mg/100 ml.

d) IgE was detected in five specimens (all Type I) as discrete coarse granules.

Fibrogen was found in 37 specimens from which 26 showed glomerular deposits only. These were mainly epithelial and were demonstrated in Types IIb, III and IV, occasionally assuming a crescent appearance.

Albumin was positive in only 19 cases ; 12 cases showed tubular involvement only.

C3 deposits usually involved the three main structures of the kidney. They involved the glomerulus alone in 20 instances. Deposits were coarsely granular, usually focal but also diffuse, especially in Type II, similar to or even more widespread than were the immunoglobulins. In seven biopsy specimens, dense C3 deposits were observed in the glomeruli with an absence of immunoglobulins. C3 deposits were found in the tubules together with IgG and M in cases excreting antiworm antibodies in their urine.

C4 deposits were found in 32 out of 46 specimens studied. Deposits were finely granular, deposited along the capil-

lary wall and restricted to the glomerulus. They were accompanied by deposits of immunoglobulins and mostly occurred in Types IIa and b.

C3PA was tested for in 28 specimens and was positive in 22. In seven cases C3PA deposits were associated with an absence of immunoglobulins ; they were mostly found in Types II and III. Tubular deposits were finely granular.

Schistosome worm antigen deposits were found in 30 out of 50 cases studied. Glomeruli only were involved in 14 cases ; vascular involvement occurred in 14 cases and tubular involvement, negative for deposits in the glomerulus, occurred in two cases. These pathological changes were of Types III and IV. The antigen appeared as coarse or fine discrete granules either focally or diffusely. Patients had a moderate or poorly selective proteinuria. Circulating antibodies were detected in 13 cases ; no circulating antigen was found.

Staining for streptolysin, hepatitis B antigen, Escherichia coli and malaria was negative.

Elution studies

Elution with citric acid buffer (pH 2.5-2.8) was performed on six specimens with positive schistosome antigen. The supernatant was studied, after concentration, for antibody and antigens by counterimmuno-electrophoresis. Specific antiworm antibodies were detected in two cases and antigens in four of the eluates. Restaining of the sections after elution did not show any deposits.

Conclusion

A strongly suspected immunological glomerular damage complicates Schistosoma haematobium infestation. The condition is always associated either by way of the classical path or by an alternate pathway, or by both. Immune complexes of various sizes could account for the mesangial or capillary wall lesions found in that type of nephritis. Even though the number of eluted specimens is still small, elution studies provide support in favour of a positive role of Schistosoma haematobium antigens in the precipitation of glomerular injury.

ETUDE DES RELATIONS IMMUNOLOGIQUES HOTE-PARASITE DANS LA BILHARZIOSE :
UTILISATION DE LA TECHNIQUE D'IMMUNOFLUORESCENCE INDIRECTE

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Jusqu'à présent, la technique d'immunofluorescence (I.F.) a surtout été utilisée en tant que moyen de diagnostic immunologique des affections bilharziennes.

Dans le cadre de nos études sur les relations hôte-parasite au cours des affections à Schistosomes, nous nous sommes proposé d'utiliser cette méthode pour préciser certaines relations immunologiques existant entre les différents stades évolutifs des Schistosomes et leurs hôtes: vertébrés et invertébrés.

Cette étude a pour but :

1) l'analyse des mécanismes adaptatifs d'un Schistosome à son hôte et particulièrement des mécanismes immunologiques, plus spécialement l'acquisition des antigènes de l'hôte par les membranes du parasite

2) la détermination de la localisation des différents antigènes

3) l'étude de certains phénomènes d'immunopathologie.

Nous avons travaillé avec une souche de bilharzie entretenue sur planorbe *Biomphalaria glabrata,* origine Porto-Rico, et hamster ou souris. La technique d'immunofluorescence utilisée a été la méthode indirecte avec contre-coloration de contraste au Bleu Evans.

Les antigènes figurés[1] ont été examinés sur coupes histologiques ou en tubes en milieu liquide.

Les coupes histologiques, faites soit à congélation au cryostat, soit par la technique de Flye Ste-Marie après inclusion à la paraffine, ont intéressé

— des adultes dans le foie d'un hôte mammifère

— des sporocystes dans les tissus du mollusque.

Les stades évolutifs qui ont été étudiés sont :

— les miracidiums libres ou dès leur pénétration

— les sporocystes primaires, secondaires et les métasporocystes

— les cercaires : ébauche sur coupes d'hépato-pancréas de planorbe, cercaires libres

— les schistosomules libres

— les adultes de bilharzies sur coupes de foie de mammifère.

Immunserums utilisés en immunofluorescence

Des lapins sont immunisés au moyen d'injections intraveineuses à raison de 3

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(1) C'est à dire antigènes soit fixés soit directement traités au stade vivant, Réd.

injections par semaine de 1 ml contenant 10 mg d'extraits antigéniques en poids sec. Une injection de rappel est pratiquée 8 jours après la 9ème injection.

Extraits antigéniques

Nous nous sommes efforcés d'obtenir des préparations antigéniques présentant des propriétés constantes et non souillées par des tissus voisins lorsqu'il s'agissait d'extraits d'un organe donné.

Les techniques de préparation sont les méthodes classiques d'extraction en solution tamponnée à basse température. Ont ainsi été préparés :

— extrait total de planorbe

— extrait d'hépato-pancréas

— extrait de membranes d'hépato-pancréas.

L'obtention d'extraits de membranes d'hépato-pancréas a été réalisée par une séparation des organites cellulaires en gradient de densité de saccharose, en centrifugeuse Beckman. On retrouve 6 bandes solides plus ou moins colorées. La fraction comprenant les membranes situées à l'interphase 37/41% est recueillie et servira comme extrait antigénique.

Le miracidium

(Libre 159 × 62μ — fixé 73 × 36 μ).

Le revêtement cuticulaire porte les cils. Ceux-ci demeurent visibles 24 h. après la pénétration du miracidium ainsi que les glandes céphaliques. Le tégument externe a une structure trilaminaire.

Le miracidium se transforme en sporocyste primaire en 24 h. D'abord de forme amoeboïde il s'allonge, forme un sac qui s'enroule en formant de nombreuses circonvolutions.

En immunofluorescence, les miracidiums ont une réactivité inconstante. Les images obtenues sont souvent très belles; la fluorescence s'établit à la limite de la cuticule et les cils sont fortement fluorescents.

Les difficultés viennent de ce que :

— une fluorescence non spécifique peut apparaître. Elle signale une souffrance quelconque du miracidium avant son utilisation qui permet la diffusion et la fixation non spécifique du fluorochrome

— par contre, si le miracidium est par trop altéré, la fluorescence ne se produit plus avec du sérum positif. Il est nécessaire d'avoir des structures intactes aux sièges des sites antigéniques spécifiques pour obtenir une bonne fluorescence.

En conséquence, il est de règle d'apprécier la fluorescence du miracidium sur des individus fraîchement recueillis. Pour donner un résultat positif, il est nécessaire d'avoir plus de 60% d'individus fluorescents.

Traités par des immunsérums provenant de leurs hôtes : mammifère ou mollusque, les miracidiums ne présentent aucune fluorescence. Au sortir de l'œuf ils ne possèdent donc pas un antigène commun avec l'un ou l'autre de leur hôte.

Par contre, traités avec un immunsérum anti adulte, les miracidiums présentent une très forte fluorescence. La cuticule et les cils sont très brillants.

Le miracidium possède donc bien un déterminant antigénique spécifique des Schistosomes.

Stades Evolutifs Retrouvés dans les Tissus du Mollusque

Sporocystes primaires

24 h. après la pénétration le miracidium prend le nom de sporocyste-mère ou

sporocyste primaire. Il est constitué par un simple sac immobile dont la taille s'accroît rapidement. A un moment donné, plusieurs cellules germinales se groupent, forment une morula et évoluent en sporocyste secondaire.

En même temps que cette prolifération cellulaire, une nouvelle membrane apparaît. C'est le feuillet pariétal qui se forme à la surface interne de l'enveloppe du sporocyste ; il varie en épaisseur, est souvent discontinu et s'épaissit au fur et à mesure du vieillissement du sporocyste. Ce dernier, traité par des sérums anti mollusque ou anti hôte mammifère, n'est pas fluorescent. Une fluorescence beaucoup plus faible que celle notée chez le miracidium peut apparaître avec des sérums anti adultes le long des revêtements membranaires des amas de cellules germinales à l'intérieur du sporocyste primaire. Ce stade évolutif semble être celui qui possède le moins de déterminants antigéniques spécifiques.

Sporocystes secondaires

Les divisions successives des cellules germinatives conduisent à la formation de sporocystes secondaires. Ceux-ci sont recouverts d'une paroi relativement mince à la surface de laquelle on peut parfois mettre en évidence des épines. On n'y trouve jamais d'embryon de cercaire. Les sporocystes secondaires mûrs émergent du sac constitué par le sporocyste primaire en déchirant et fin tégument externe. Ces sporocystes secondaires migrent alors vers l'hépato-pancréas, lieu privilégié de la phase finale de leur développement. Certains peuvent prendre des voies anormales mais dans ce cas ne donnent pas naissance à des cercaires.

Une fois en place dans l'hépato-pancréas, les sporocystes secondaires prennent le nom de métasporocystes.

Les sporocystes secondaires âgés à l'intérieur des sporocystes primaires ou en migration peuvent porter un très fin liséré fluorescent s'ils sont traités avec des sérums anti adultes mais sont négatifs avec tous les autres immunsérums essayés.

Métasporocystes

C'est le stade larvaire final immobilisé dans son dernier habitat dans les tissus du mollusque à l'intérieur duquel les cercaires vont évoluer.

Les ébauches de cercaires se différencient à partir de masses germinales et sont visibles 16 à 18 jours après l'infestation c'est-à-dire 2 à 4 jours après l'arrivée des sporocystes secondaires dans la glande. La forme cercarienne apparaît vers le 25ème jour et atteint son plein développement à 32 jours. L'émission débute au 39ème jour.

Avec des sérums anti hôte mammifère on n'obtient aucune fluorescence. Avec des sérums anti adultes les ébauches de cercaires fixent les anticorps anti adultes même à l'intérieur des métasporocystes avec une intensité d'autant plus forte qu'elles sont plus âgées. La localisation de la fluorescence est membranaire. Dans les sporocystes primaires ou secondaires qui ne contiennent pas encore d'ébauches de cercaires, aucune formation ne présente de fluorescence avec un tel immunsérum. Avec un sérum anti mollusque, une assez forte fluorescence de membrane est visible.

Les métasporocystes dont le revêtement externe est, à certains moments de leur développement, en contact très intime avec le tissu de la planorbe, doivent acquérir de ce fait des déterminants antigéniques communs, sans doute par remaniement moléculaire des composants de leurs membranes.

Il apparaît que cela ne serait pas au cours de la migration des sporocystes à l'intérieur de tissu de l'hôte que se produirait cette acquisition, mais seulement au moment où les échanges entre les larves et les tissus du mollusque sont devenus très actifs et intimes. Des interpénétrations tissulaires avec échange de matériel mises en évidence par microscopie électronique paraissent être à la base de ces acquisitions qui permettent aux sporocystes de ne plus être reconnus comme corps étrangers mais au contraire tolérés comme formant une entité avec les tissus de l'hôte. Cette adaptation tégumentaire permet au parasite de résister à l'action des secrétions des leucocytes des mollusques.

Développement du Tegument de la Cercaire

La cercaire doit s'adapter à une série de milieux différents entraînant des changements profonds dans son revêtement externe. Elle se développe dans le mollusque à l'abri, en tant qu'embryon, à l'intérieur du métasporocyste. C'est ce dernier qui, à ce moment, constitue le parasite de la planorbe. La cercaire «naît» alors dans l'environnement interne du mollusque. Elle émerge ensuite dans l'eau, milieu totalement dépourvu d'éléments nutritifs. Elle s'y comporte comme un organisme libre et ne récupère son état parasitaire qu'après la pénétration dans les tissus d'un hôte mammifère. La cercaire a donc rencontré 3 éconiches en 3 jours en moyenne dans les conditions normales.

Les embryons de cercaires à l'intérieur du métasporocyste ont un revêtement externe (épithélium primitif) issu des cellules de la paroi du métasporocyste. Au fur et à mesure de la croissance des ébauches cercariennes, ce dernier disparaît pour faire place à un revêtement trilaminaire présent sur les téguments de la cercaire au moment de l'émission. Ce dernier est formé à partir des cellules somatiques de l'mbryon cercarien. Celles-ci viennent former un nouveau feuillet sous l'épithélium primitif qui, parallèlement, dégénère et disparaît.

Il est aisé de comprendre, selon ce schéma, que les stades évolutifs successifs des cercaires sont des organismes très différents d'un point de vue structural, métabolique et par là même immunologique.

Traitées par les immunsérums anti hépato-pancréas, les cercaires présentent une vive fluorescence sur toute la surface de leur corps : la tête et la queue. Seule la cuticule est fluorescente ; elle apparaît sous la forme d'un trait fin très nettement délimité entourant la masse des tissus intérieurs. Ce trait a la même brillance autour de la queue qu'autour de la tête ; il ne s'interrompt qu'au niveau de l'orifice des glandes céphaliques. Les immunsérums anti hépato-pancréas total ou anti membranes donnent des images identiques ; elles sont cependant plus nettes avec les sérums anti membranes. Cet aspect est différent de celui observé après action sur les cercaires de sérum de bilharziens.

La cercaire, à son émission, porte donc un caractère antigénique «planorbe» qui n'est pas présent sur le miracidium avant sa pénétration dans le mollusque.

Les schistosomules

Les cercaires sont transformées en schistosomules en 15 minutes. Elles atteignent le poumon en 7 à 9 jours en moyenne (au plus tôt en 4 jours). Puis elles migrent jusqu'aux veines portes du foie. Les adultes matures accouplés apparaissent entre le 26ème et le 30ème jour.

Traités par des sérums anti adultes les schistosomules peuvent présenter une forte fluorescence, plus marquée le long du tractus intestinal et sur la cuticule.

Certaines cellules des tissus de l'hôte au voisinage des larves peuvent également présenter une certaine fluorescence due aux produits métaboliques des vers.

Avec des sérums anti souris ou anti hamster une fluorescence est mise en évidence le long de la cuticule externe. Aucune fluorescence n'est obtenue avec des sérums anti mollusques.

Les schistosomules, immédiatement après leur obtention par passage à travers la peau de souris, ne sont pas colorées par les immunsérums anti hépato-pancréas et anti membranes cellulaires. Le caractère antigénique «planorbe» est donc perdu lorsque la tête de la cercaire après s'être détachée de la queue, se dépouille de son enveloppe extérieure en pénétrant dans l'hôte mammifère. Ce fait confirme la localisation superficielle de l'antigène «planorbe».

Les schistosomules adultes

Avec des sérums anti souris l'immunofluorescence permet la détection préférentielle des antigènes somatiques de la cuticule. Certaines localisations électives sur le tractus digestif laissent soupçonner l'intervention d'antigènes métaboliques. La réactivité est identique chez les mâles et les femelles mais l'utérus et son contenu, œufs et granules vitellins, sont le siège, dans cet organe, de fluorescences non spécifiques. Comme pour les schistosomules âgées des résultats négatifs sont obtenus avec des immunsérums anti mollusques.

Les schistosomules et les schistosomes adultes portent donc à leur surface un antigène spécifique d'espèce plus un antigène commun à l'hôte qui les héberge

mais pas d'antigène voisin de ceux des planorbes.

Les œufs

Ils présentent toujours une fluorescence non spécifique. Certains granulomes formés autour des œufs, par contre, peuvent présenter une sorte de pointillé fluorescent constitué de fins granules correspondant à des zones de nécrose. Ce phenomène parait dû à la présence d'immuncomplexe mis en évidence par une réaction positive en immunofluorescence plus qu'à l'action d'antigène libéré par l'œuf et se répandant dans les tissus environnants.

Conclusion

En conclusion, nous avons pu démontrer que chez le mollusque comme lors de leur passage chez l'hôte vertébré, les larves de schistosomes acquièrent des caractères antigéniques communs à ceux de l'hôte qui les héberge.

Les cercaires à leur sortie du mollusque portent un déterminant antigénique du type «planorbe». Ce déterminant antigénique n'est pas présent sur le miracidium. Par contre, nous l'avons mis en évidence sur les parois des métasporocystes et sur certaines formations contenues à l'intérieur de ces sporocystes qui sont les ébauches de cercaires.

Certaines travaux antérieurs avaient déjà montré l'existence de déterminants antigéniques communs entre le parasite et son hôte, mais ceci par des techniques utilisant des antigènes bruts solubles. Ici, le caractère mis en évidence paraît lié à des remaniements moléculaires des membranes externes et doit être acquis lors d'échanges entre le parasite et le tissu de son hôte.

Ces premiers résultats ont fait apparaître que, comme dans le cas des stades des schistosomes évoluant dans les

tissus des mammifères, une grande partie des réactions immunologiques des stades larvaires retrouvées chez le mollusque correspondaient à des phénomènes de membranes. Les remaniements importants des surfaces externes de ces stades évolutifs successifs : miracidiums, sporocystes primaires, secondaires, métasporocystes, cercaires, s'accompagnent de modifications immunologiques.

Dans une dernière série de travaux nous avons recherché la nature chimique de ces antigènes de membranes.

Les antigènes de planorbes portés par les cercaires et les sporocystes-filles sont constitués, au moins en partie, par un haptène lipidique provenant des membranes cellulaires de l'hépato-pancréas de mollusque.

La recherche de l'identification des lipides responsables est en cours.

IMMUNODIFFUSION STUDIES ON DEVELOPMENTAL STAGES OF SCHISTOSOMA MANSONI

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For immuno-electrophoretic comparison of antigenic components in different developmental stages of Schistosoma mansoni we used separate extracts from adult male and female S. mansoni worms collected from white mice, extracts from eggs, miracidia, and cercariae derived from Biomphalaria alexandrina and Biomphalaria glabrata. The corresponding antisera were prepared in rabbits by use of complete Freund's adjuvant.*

As far as the homologous reactions are concerned the numbers of precipitin arcs we obtained with material from male and female adult worms, eggs and cercariae were similar to those described by Capron et al. (1965). Additionally we also worked with miracidia and the respective antiserum. We were able to confirm the results of Capron, who found no essential antigenic difference between adult female and male worms.

A variety of cross-reactions could be found too when antigen and antiserum against miracidia was studied against antigenic extracts of different developmental stages of S. mansoni. The cross-reactivity with anti-cercarial sera is surprisingly low (Figs. 1, 2).
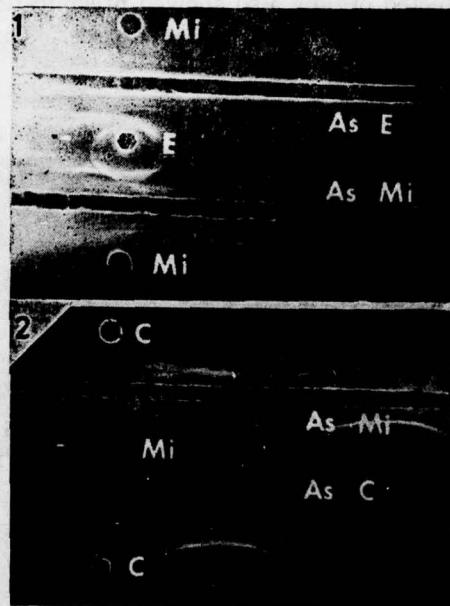
Fig. 1. Slide showing clear immuno-electrophoretic heterologous cross-reaction between S. mansoni eggs and miracidia. Mi, miracidium antigen, As Mi, anti-miracidium serum, E, S. mansoni egg antigen; As E, anti-egg serum.

Fig. 2. Immunoelectrophoresis of heterologous reaction between S. mansoni miracidia and cercariae, demonstrating clear cross-reaction. C, cercarial antigen (B. glabrata); As C, anti-cercarial serum; (Mi, As Mi, see Fig. 1).

Capron was the first to study the immunological reactions between the parasite and its intermediate host. Unlike Capron, who used the whole body of the snails, we only took the hepatopancreas of infected and non-infected *B. alexandrina* and *B. glabrata,* and used the extracts for immunoelectrophoretic studies.

We found that not only antigens from infected organs gave rise to many cross-reactions with antisera against worms, cercariae, etc., which was to be expected (Fig. 3), but extracts from non-infected organs also showed precipitation arcs, though to a minor degree. One,

respectively 2, distinct lines can easily be seen when various antigens are plotted against antiserum from non-infected hepatopancreas (Fig. 4).

Table 1 sums up all results from cross-reactions experiments.

Based on these results we tried to find out whether these cross-reacting antigens from non-infected snails would be suitable for vaccination experiments in mice. Injection of hepatopancreas prior to challenge could lead to a reduction of the worm burden. The results from these experiments are communicated in another paper .
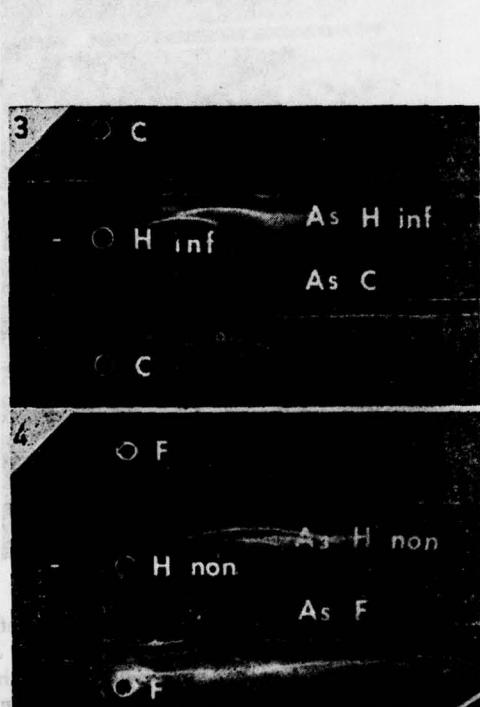
Fig 3. Heterologous immunoelectrophoresis reaction of *S. mansoni* cercariae and infected snail hepatopancreas (*B. glabrata*) with clear cross precipitins H. inf, hepatopancreas antigen from *B. glabrata* infected with *S. mansoni;* As H inf, antiserum against hepatopancreas from *B. glabrata* infected with *S. mansoni;* (C, As C, as in Fig. 2).

Fig. 4. Immunoelectrophoresis of heterologous reaction between female *S. mansoni* worms and non-infected snail hepatopancreas (*B. glabrata*) clearly demonstrating cross-reaction F, adult female *S. mansoni* antigen; As F, antiserum against adult female *S. mansoni.* H non, hepatopancreas antigen from noninfected *B. glabrata:* As H non, antiserum against hepatopancreas from noninfected *B. glabrata*

TABLE 1. Cross-reaction of hepatopancreas (HP) of *Biomphalaria* snails with *Schistosoma mansoni* antigens. Number of precipitin arcs in immunoelectrophoresis using anti-HP-sera (I) and anti-stage-specific-sera (II)

Organ or Stage	I				II			
	B. glabrata		*B. alexandrina*		*B. glabrata*		*B. alexandrina*	
	AS inf.	AS non-inf.	AS inf.	AS non-inf.	HP inf.	HP non-inf.	HP inf.	HP non-inf.
HP inf.	22	15	23	6	22	15	23	6
HP non-inf.	15	18	9	14	15	18	9	14
Egg	2	1	8	0	6	0	4	0
Miracidium	1	0	1	0	7	4	10	4
Cercaria	14	2	10	1	7	8	7	9
Female worm	12	2	10	2	8	3	6	2
Male worm	10	1	6	1	7	3	4	2

AS — antiserum

LITERATURE CITED

Capron, A., Biguet, J., Rose, F. & Vernes, A., 1965. Les antigènes de Schistosoma mansoni. I. Etude immunoélectrophorétique comparée de divers stades larvaires et des adultes de deux sexes. Aspects immunologiques des relations hôte-parasite de la cercaire et de l'adulte de S. mansoni. Ann. Inst. Pasteur, 109: 798-810.

PROTECTION TRIALS WITH NON-INFECTED SNAIL HEPATOPANCREAS IN MICE

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Immunological crossreactions between constituents of snails and various developmental stages of schistosomes, as determined in the immunoelectrophoresis system, are known since 1965 due to the work of Capron et al. (1965). Repeating this work, not with whole snail extracts but with extracted hepatopancreas from non-infected *Biomphalaria glabrata* snails, we have also been able to demonstrate an immunological relationship between this material and extracts from adult worms of both sexes. The same finding was also very clear with cercariae (Figs. 1, 2).

As can easily be seen, at least one precipitin line is shared if either *Schistosoma* antisera and the hepatopancreas antigen are used or if antisera against hepatopancreas are plotted against adult worm antigens.

Whether or not this finding plays any role in the invasion and proliferation mechanism of the miracidium in the snail is purely speculative, because our knowledge of «immunity processes» in snails is poor and we do not know whether invertebrates show the same immune tolerance phenomena that mammals do.
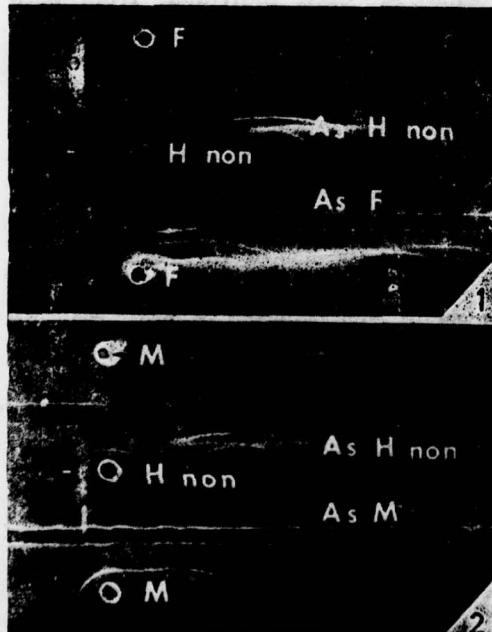
Fig. 1. Heterologous immunoelectrophoretic reaction between adult female *Schistosoma mansoni* worms and non-infected snail hepatopancreas (*Biomphalaria glabrata*) clearly demonstrating cross-reaction

F, adult female *S. mansoni* antigen; As F, antiserum against adult female *S. mansoni*; H Non, hepatopancreas antigen from non-infected *B. glabrata*; As H non, antiserum against hepatopancreas from non-infected *B. glabrata*.

Fig. 2. Heterologous immunoelectrophoretic reaction between adult male *Schistosoma mansoni* worms and non-infected snail hepatopancreas (*Biomphalaria glabrata*) clearly demonstrating cross-reaction

Labelling as in Fig. 1. except that M (Male) is substituted for F (Female).

TABLE. 1. Immunization of mice with non-infected hepatopancreas from
Biomphalaria glabrata.

No. of mice	Immunization with (mg)	Dissection in days after challenge	Deaths	Worms collected			Average worms per mouse
				Male	Female	Total	
100	—	40	44	808	552	1360	24.3
100	2 × 0.25	40	8	664	372	1036	11.3
100	—	40	16	1248	920	2168	25.8
100	2 × 0.25	40	28	644	408	1052	14.6
100	—	60	48	812	716	1528	29.4
100	2 × 0.25	60	56	332	228	560	12.7
100	—	60	56	608	580	1188	27.0
100	2 × 0.25	60	32	852	558	1440	21.1

Nabih et al. (1974) observed that injections of extracts from chemically pretreated snails into mice had a marked effect against infection with *S. mansoni*, while extracts from untreated snails failed to induce any immunotherapeutic protection. On the other hand, the cross-reactions described above have been strong enough to justify immunisation experiments with extracts from non-infected snail hepatopancreas.

Total weight of 3.75 g of hepatopancreas was collected from 120 3-months old *Biomphalaria glabrata*, minced in a tissue grinder, suspended and extracted with 15 ml saline. This antigen mixture was always prepared freshly before use.

The mice were injected twice subcutaneously with an aliquot corresponding to 250 micrograms of wet pancreas. Two weeks after the second immunisation the immunized groups and the respective controls were challenged by subcutaneous injection of 130 cercariae from

B. glabrata and the mice were dissected 40 and 60 days after challenge. Our results are shown in Table 1.

Inevitably we lost some mice due to the long time between challenge and dissection, but more mice died in the control groups than in the immunized group.

Taking into account the amount of worms in each individual from both groups, we found, according to the Chi-square test, a significant reduction of the worm burden in the immunized groups in comparison to the non-immunized animals. The 5% error calculated from the tables is 3.841 and the 1% error 6.635.

Further experimentation is planned as regards the use of various adjuvants, repeated injections at different times, and especially the isolation of cross-reacting substances from the non-infected hepatopancreas of snails, in order to clarify the protective effect in mice of pancreatic antigens against challenge with *S. mansoni* cercariae.

LITERATURE CITED

Capron, A., Biguet, J., Rose, F. & Vernes, A.,
1965. Les antigènes de Schistosoma mansoni.
I. Etude immunoélectrophorétique comparée
de divers stades larvaires et des adultes de
deux sexes. Aspects immunologiques des rela-
tions hôte-parasite de la cercaire et de l'adulte
de S. mansoni. Ann. Inst. Pasteur, 109: 798-810.

Nabih, I., Akeson, A. & Theorell, H., 1974. Im-
munological and immunotherapeutical control
of schistosomiasis infection through extracts
from chemically pretreated snails. Third inter-
nat. Congr. of Parasitology, Munich, 25-31 Aug.
1974, Proceedings, 2: 829.

STUDIES ON THE SUITABILITY OF THE REFERENCE SKIN-TEST ANTIGEN FOR THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF SCHISTOSOMIASIS HAEMATOBIA

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Several attempts have been made in the past to extract an antigen from adult schistosome worms or their cercariae to be utilised in the diagnosis of bilharziasis by means of the complement fixation test. Early attempts by Yoshimoto (1910) and Fairley (1919) did not give completely satisfactory results. Taliaferro et al. (1928) observed that substances responsible for false precipitin reactions with syphilitic serum could be extracted with organic solvents from cercarial concentrates stemming from snail hepatopancreas. Antigens made from adult *Schistosoma* worms were introduced by Khalil & Hassan (1932), by preparing saline extracts of ether-extracted *S. bovis* worms for skin tests. The first successful method of extraction of an antigen suitable for the complement fixation test was published by Chaffee et al. (1954). The antigen adapted by them was a buffered extract of ether-extracted schistosomes, which had been immediately preserved by desiccation from the frozen state, extraction being done from dried tissue under conditions of time and temperature less likely to be associated with destructive changes.

Facilities for the preparation of such antigens are not always available, and the need for ready-made antigen present-ed itself each time we had to perform the complement fixation test. By means of preliminary trials, we ascertained that the WHO skin reference antigen might meet our requirements. The reference skin-test antigen is a sterile adult worm acid-soluble protein fraction of *S. mansoni* or *S. japonicum* adjusted to 0.03 mg N/ml. Merthiolate has been substituted for phenol in Coca's solution (WHO, 1965a, p. 216).

The work here described was conducted at the Medical Research Centre in Baghdad, Iraq, on patients all suffering from *S. haematobium* infection, which is the only species affecting man in Iraq.

Materials and Methods

The complement fixation test (CFT) using the reference skin-test antigen was carried out on sera of children and adults suffering from schistosomiasis haematobia, in order to evaluate the activity of the antigen. The test itself was carried out quantitatively according to the recommendations of the WHO Scientific Group on Research in Bilharziasis (WHO, 1965b). The dilution of the antigen used by us in the test was 1 in 10.

Schistosome ova were detected after centrifugation of a specimen of urine ob-

tained after morning activity, i.e., at 11 a.m., in order to ensure the excretion of the greatest number of eggs possible. The deposit of the urine after centrifugation was examined microscopically according to standard parasitological methods.

In a certain number of cases, the circumoval precipitin (COP) test was conducted according to the method des-

cribed by Oliver–Gonzalez (1954), so as to compare between the results of both these serological tests.

Results and Discussion

The present work was conducted on 238 individuals all with a history of bilharziasis and living in the endemic area. The results recorded in Tables 1 and 2

TABLE 1. Results of the complement fixation test (CFT) in the detection of schistosomiasis haematobia using WHO reference antigen as compared to those obtained by the circumoval precipitin (COP) test.

Age group years	No. of patients	Clinical history of of schistosomiasis +	Clinical history of of schistosomiasis %	No. of patients passing ova of S. h. in urine +	No. of patients passing ova of S. h. in urine %	No. CFT* +	No. CFT* %	No. COP** AC***	No. COP** +	No. COP** %
6 — 10	57	57	100.0	51	89.4	45	78.9	4 (7%)	52	91.0
11 — 15	67	65	97.0	65	97.0	60	89.5	3 (4.47%)	64	95.5
16 — 20	29	24	82.7	17	58.6	20	68.9	2 (6.8%)	24	82.7
21 — 25	14	13	92.8	7	50.0	10	71.4	2 (14.2%)	12	85.7
26 — 30	19	16	84.2	5	26.3	13	68.4	2 (10.5%)	14	73.7
31 — 35	10	8	80.0	4	40.0	9	90.0	0	6	60.0
36 — 40	10	9	90.0	3	30.0	10	100.0	0	9	90.0
41 — 45	4	2	50.0	2	50.0	2	50.0	0	2	50.0
46 — 50	10	8	80.0	3	30.0	7	70.0	0	7	70.0
51 — 55	5	5	100.0	1	20.0	4	80.0	0	3	60.0
56 — 60	4	2	50.0	2	50.0	2	50.0	0	2	50.0
60	9	5	55.5	2	22.2	4	44.4	0	5	55.5
Total	238	214	89.0	162	68.0	186	78.0	13 (5.4%)	200	84.0

* Using WHO reference skin antigen

** Using egg antigen

*** AC = anticomplementary activity

TABLE 2. Results given in Table 1 regrouped as to age-groups.

Age group years	No. of patients	Clinical history of schistoso- miasis		No. of patients passing ova of S. h. in urine		CFT				COP	
		+	%	+	%	+	%	AC	%	+	%
< 11	57	57	100.0	51	89.4	45	78.9	4	7	52	91.0
11 — 20	96	89	92.7	82	85.4	80	83.3	5	5.2	88	98.9
21 — 30	33	29	87.8	12	36.3	23	69.7	4	12.0	26	78.8
31 — 40	20	17	85.0	7	35.0	19	95.0	0	0	15	75.0
> 40	32	22	68.7	10	31.0	19	59.4	0	0	19	59.4
Total	238	214	89.0	162	68.0	186	78.0	13	5.4	200	84.0

demonstrate that in the 6-15 year age-group more ova-positive individuals are revealed by urine examination (i.e. 116) than by CFT (105), which represents a statistically significant difference. In the same age group the COP test gave results equal to urinary examination. In the older, i.e. the 16-60 year age groups, the picture was reversed, i.e. more cases were positive by the serological tests than by the urinary examination. There is a decline in the positive results given by all three tests in the older age groups (from 16-60 years) as compared to the younger age groups (6-15 years old). Considering the total number of cases (238) comprising all age-groups, ova were detected in the urine of 162 patients (68%), the CFT was positive in 186 patients (78%) and the COP test was positive in 200 (84%) of the patients.

We selected our patients in the urological outpatient department of the Medical College Hospitals in Baghdad, from among individuals living in neighbouring endemic areas and complaining of definite clinical manifestations of urinary schistosomiasis, particularly terminal haematuria, burning micturition and with a definite clinical history of the disease. This record of the clinical history of the subjects, including the discovery of bilharzial ova in urine or treatment with antischistosomal drugs, was found valuable and even necessary, because cooperation of patients and their follow-up were not satisfactory. Repeated urine examination for ova was not possible in all patients. For this reason we have confined our evaluation of the reactivity of sera to the CFT, using the reference antigen, to the groups of patients in whom a definite diagnosis was secured by finding S. haematobium ova in urine (Table 3).

The CF test (Table 3) gave positive reactions in 81.6% of the sera of haematobiasis patients in the 6-15 year age group, while the COP test gave 95.4% in the same age group. Previously, however, Chaffee et al. (1954) had recorded positive results in 98.4% of patients suffering from S. mansoni, i.e. they obtained

TABLE 3. Comparison of the CFT and the COP test in the young age groups positive for *Schistosoma haematobium* ova in the urine.

Age group years	No. of patients postive for S. haematobium ova in urine		CFT positive patients		Anticomplementary activity		COP positive patients	
	No.	%	No.	%	No.	%	No.	%
6 — 10	48	100	33	68.8	3	6.3	43	89.6
11 — 15	61	100	56	91.8	4	6.5	61	100.0
Total	109	100	89	81.6	7	6.4	104	95.4

higher results than our own. Jachowsky & Anderson (1961) reported that the CFA* test showed the greatest specificity (94%) in patients suffering from *S. mansoni* infections and that the COP test was only slightly less specific (91%) ; however, it provided a procedure by which clinicians could screen patients. Oliver-Gonzalez et al. (1955a) stated that COP reaction was species-specific, with minor cross-reactions between *S. mansoni* and *S. haematobium*.

For the performance of the COP test, we used *S. haematobium* eggs as antigen. This, presumably, is responsible for the higher percentage of COP positives in our series as compared to those of Oliver-Gonzalez et al. (1955b) and Jachowsky & Anderson (1961), who tested the specificity of reaction in sera of *S. haematobium* patients using *S. mansoni* eggs.

With regard to non-specific reactions Jachowsky & Anderson (1961) found only two sera among those of 485 patients examined by them, who reacted to car-diolipin antigen. Both patients were stool positive for *S. mansoni* eggs and were serologically reactive in all serological

tests for schistosomiasis. Our control tests proved negative.

Since trichinosis has not been found in Baghdad or neighbouring endemic areas, reactions observed in our studies can probably not be attributed to this disease. Anticomplementary activity took place in 5.4% of sera of 238 schistoso-miasis patients subjected to the comple-ment fixation test (Tables 1, 2). This is most likely due to the incomplete purifi-cation of the reference skin antigen. Rie-ber et al. (1961) reported that exhaustive ether extraction in the purification of the antigen they produced for the comple-ment fixation test effectively removed anticomplementary substances, as well as the worm components responsible for cross-reactions with syphilitic sera.

The clinician concerned with indivi-dual patients requires methods which will confirm his diagnosis and enable him to follow up the treatment of his patients. Similarly the public health epidemiolog-ist needs to study the necessary epide-miological parameters in the younger segment of the population in order to evaluate the effect of control measures.

* Adult S. mansoni (Chaffee's) antigen used for the CF test.

Such procedures should be specific and should become non-reactive when the infections are eliminated. However, the CF test and the COP test can be employed in screening large numbers of persons, although repeated examinations of urine and stools will also be needed in non-reactive cases. The COP test is of particular interest since it is easy to conduct and highly specific. Moreover, according to Oliver-Gonzalez et al. (1955c), it becomes negative a few months after successful therapy.

Summary

The complement fixation test, using the WHO reference skin antigen, was carried out on a series of sera obtained from 238 Iraqi patients, clinically diagnosed to be suffering from schistosomiasis haematobia (Tables 1 and 2). For the sake of comparison and evaluation of the CF test, urine examinations for ova and the COP test were performed for each patient.

The evaluation of the reactivity of the sera to the CFT (using the reference antigen) was confined to the age-groups of patients in whom a definite diagnosis was secured by finding ova in urine. The sera of young children were less reactive than those of the older age groups. The CFT gave positive reactions in 68.8% of the sera taken from the 6-10 year age group and 91.8% in the 11-15 year age group (Table 3). The COP test gave higher results, i.e. 89.6% in the former age group and 100% in the latter.

Sera of our patients infected with S. haematobium gave lower results in the CFT than those obtained by Chaffee et al. (1954) and Jachowsky & Anderson (1961) in patients suffering from S. mansoni infection. This might be due to a difference in specificity between the two infections, since the reference antigen used by us in sera of patients suffering from schistosomiasis haematobia is also derived from adult S. mansoni worms. Also, anti-complementary reactions occurring in CF tests in our series (5.4%) may indicate incomplete purification of the reference antigen.

Whatever might be said in favour of the immunodiagnostic tests, microscopic urine examination has proved to reveal the highest percentage of positive results in the young age groups. Repeated examinations of urine and stool will also be needed in non-reactive cases.

LITERATURE CITED

Chaffee, E.F., Bauman, P.M. & Shapilo, J.J., 1954. Diagnosis of schistosomiasis by complement fixation. Amer. J. trop. Med. & Hyg., 3: 905.

Fairley, N.H., 1919. The discovery of a specific complement fixation test for bilharziasis and its practical application in clinical medicine. J. roy. Army med. Corps, 32: 449-460.

Jachowsky, L.A. & Anderson, R.I., 1961. Evaluation of some laboratory procedures in diagnosi, ; infections with Schistosoma mansoni. Bull. W.d Hlth Org., 25: 675-693.

Khalil, M. & Hassan, A., 1932. A preliminary note on a new skin reaction in human schistosomiasis. J. Egypt. med. Assoc., 15: 129-130.

Oliver-Gonzalez, J., 1954. Anti-egg precipitins in the serum of humans infected with Schistosoma mansoni. J. infect. Dis., 95: 86-91.

Oliver-Gonzalez, J., Bauman, P.M. & Benson, A.S., 1955a. Immunological aspects of infections with Schistosoma mansoni. Amer. J. trop. Med. Hyg., 4: 443-454.

Oliver-Gonzalez, J., Bauman, P.M. & Benson, A.S., 1955b. Species specificity of the anti-egg precipitin in schistosomiasis serums. J. infect. Dis., 96: 95-100.

Oliver-Gonzalez, J., Ramanos, F.L. & Coker, C.M., 1955c. Serological reactions against egg antigens as an aid in the evaluation of therapy in

schistosomiasis. Amer. J. trop. Med. Hyg., 4: 908-912.

Rieber, S., Anderson, R.I. & Radka, M.G., 1961. Serologic diagnosis of Schistosoma mansoni infections. III. Isolation and purification of antigen from adult S. mansoni for the complement-fixation test. Amer. J. trop. Med. Hyg., 10: 351-355.

Taliaferro, W.H., Hoffman, W.A. & Cook, D.H., 1928. A precipitin test in intestinal schistosomiasis (S. mansoni). J. prev. Med. (Baltimore), 3: 395.

Yoshimoto, M., 1910. Ueber die Komplementbindungsreaktion bei der Schistosomum-Krankheit in Japan. Ztschr. f. Immunitätsforsch. u. exp. Therap., 5: 438-445.

WHO, 1965a. Snail control in the prevention of bilharziasis. World Health Organisation Monograph Series No. 50 (Geneva).

WHO, 1965b. Scientific Group on Research in Bilharziasis (Measurement of the Public Health Importance). Original: English; Report to the Director-General PA/187.65. Geneva, 9-14 August 1965.

EFFECTS OF SPLENECTOMY ON SERUM IMMUNOGLOBULINS IN SCHISTOSOMAL HEPATIC FIBROSIS

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Immunological reactions, humoral and cellular, have been found to occur in association with schistosomal hepatic fibrosis. The granuloma in the liver is mostly a cellular immune response (Von Lichtenberg, 1964 ; Warren et al., 1967 ; Ghanem et al., 1974). Humoral immunity in the form of increased serum immunoglobulins has been observed and confirmed by many authors (Ghanem, 1962 ; Antunes et al., 1971 ; Bjornebae et al., 1971; Bassily et al., 1972). Increased immunoglobulins IgG and IgM in the sera of these patients were demonstrated by Nichaux (1966), Hillyer (1969), Antunes et al. (1971) and Ghanem et al. (1973, 1974).

Ghanem, in 1962, gave the following evidence for the presence of immunological reactions in hepatic bilharziasis : (i) The presence of a rheumatoid-like activity in the serum ; (ii) a positive autoimmune complement fixation test ; (iii) band precipitation in gel diffusion test ; (iv) a dysproteinaemic electrophoretic immune pattern ; (v) plasma cell hyperplasia in myelogram ; (vi) the presence of lymphoid and plasmoid hyperplasia in the reticulo-endothelial system ; (vii) the presence of lymphocytic reaction in the liver and lymph nodes at the porta hepatis.

Moreover, Ghanem et al. (1972) have shown that cyclophosphamide therapy reduced the size of the schistosomal liver. This drug is known to affect B lymphocytes mainly, thus mainly affecting humoral immunity.

The increase of the various types of immunoglobulins in schistosomal hepatic fibrosis cases was attributed to : (i) Antischistosomal antibodies (Da Silva & Ferri, 1965 ; Kagan & Pellegrino, 1961 ; Sadun & Gore, 1970) ; (ii) increased bacterial antigen absorbed from the gut (Sherlock, 1970 ; Lehman et al., 1972 ; Ghanem et al., 1973 ; (iii) damaged liver cells (Sherlock, 1970). Trigger et al. (1972) suggested that damaged hepatic Kupffer cells fail to sequestrate bacterial antigen with a resulting increase in immune response.

The present study aims at the evaluation of a possible role of the spleen in the synthesis of excessive immunoglobulins in patients with schistosomal hepatic fibrosis.

Material and Methods

Of 20 schistosomal hepatic fibrosis patients with marked splenomegaly but no ascites, 14 were males and six were females ; their ages ranged from 16 to 43 years. The bilharzial nature of the liver affection was proved by excisional liver biopsy. These cases were all subjected to splenectomy. Before the operation, the

complete blood picture was taken and liver function tests were made ; for each patient total serum proteins were measured and their electrophoretic patterns were investigated. Blood sera of fasting patients were taken before, and 2 weeks after splenectomy for the estimation of the immunoglobulins IgG, IgM and IgA by the radial immuno-diffusion technique (Hyland Division, Travenol Laboratories, California). The study also included 12 healthy controls (4 females and 8 males) between the ages of 20 and 45 years.

Results

Significantly increased levels of IgG ($P < 0.01$) and IgM ($P < 0.05$) were found to occur in the bilharzial hepatic fibrosis patients as compared with the normal controls (Table 1). The IgA level, however, was almost unchanged ($P > 0.05$).

Following splenectomy, a statistically significant reduction in IgG was found ($P < 0.05$), the decrease being 11.1% of the presplenectomy level. The reduction in IgM and IgA was not statistically significant (Table 1).

Discussion

Enlargement of the spleen in schistosomal hepatic disease was considered to be of congestive origin (congestive splenomegaly) (Salah, 1962). However, the presence of splenic enlargement very early in the intestinal phase as well as in the early hepatomegalic phase does not support such a hypothesis (Awadalla, 1972). Aboul-Enein et al. (1972) showed that the splenic enlargement in schistosomal hepatic fibrosis is not only an index of congestion but also an expression of reticulo-endothelial hyperplasia, as cases with a low vascular index were associated with marked pulp hyperplasia. Such a proliferative change in the pulp of the spleen is an expression of host-parasite adjustment (Moore & Warren, 1967).

Homogenates of the spleen in cases of hepatic bilharziasis showed increased beta and gamma globulins in simple agar gel electrophoresis, as well as in immuno-electrophoresis, a finding which supports the possibility of local synthesis of globulins in the spleen (Ghanem et al., 1974).

TABLE 1. Serum immunoglobulins in schistosomal hepatic fibrosis (without ascites) before and after splenectomy, as compared to normal levels.

Subjects	Immuno-globulin	Immunoglobulins (mg/100 ml)		
		before splenectomy	2 weeks after	% decrease
Schistosomal	IgC	1763 ± 191 (P<0.01)*	1567 ± 534	11.1 (P<0.05)
Control		1262 ± 95		
Schistosomal	IgM	111 ± 49 (P<0.05)*	100 ± 40	9.9 (P>0.05)
Control		83 ± 28		
Schistosomal	IgA	247 ± 38 (P>0.05)*	238 ± 128	3.6 (P>0.05)
Control		233 ± 71		

* raised levels as compared to controls

In the present study, a significant rise was seen in both the IgM and IgG levels of patients as compared to the normal controls. Moreover, it was observed that removal of the spleen caused a significant reduction in the serum concentration of IgG. This finding proves that the spleen shares in the formation of immunoglobulins particularly of IgG.

This significant reduction of IgG within 2 weeks suggests that removal of the spleen may be of value in reducing humoral immunity with its subsequent biological effects.

As the liver is the main site of synthesis of IgG, splenectomy did not reduce the IgG to normal level.

LITERATURE CITED

Aboul-Enein, A., Moustafa, F., Massoud, G., Helmy, A. & El-Sallab, R., 1972. Observations on intrasplenic vasculature in schistosomiasis and non-congestive splenomegaly. J. trop. Med. Hyg., 75(4): 67.

Antunes, L.J., Reis, A.P., Pellegrino, J., Taveres, C.A. & Kate, N., 1971. Immunoglobulins in human S. mansoni. J. Parasit., 57: 539.

Awadalla, N.H., 1972. Histopathological study of liver, lung and large intestine of laboratory animals experimentally infected with Schistosoma mansoni. M.D. Thesis. Fac. Med., Univ. of Alexandria.

Bassily, S., Higashi, G.I., Farid, A. & Williams, R.E., 1972. Serum immunoglobulins in schistosomiasis mansoni. J. trop. Med. Hyg., 75(4): 73.

Bjornebae, M., 1971. Anti-Salmonella agglutinins in chronic active liver disease. Lancet, 2: 484.

Da Silva, L.C. & Ferri, R.G., 1965. Immuno-diffusion studies in human S. mansoni. II. Localisation of antibodies by immuno-electrophoresis. Rev. Inst. Med. Trop. São Paulo, 7: 7.

Ghanem, M.H., 1962. The R.A. test in schistosomal liver fibrosis. Amer. med. J., 8: 195.

Ghanem, M.H., Guirgis, F.K., El-Sawy, M., Khalil, S., Aboul-Kheir, F., Mourad, A.S. & Ismail, A., 1975. Study of some interplaying immunological factors in bilharzial hepatic fibrosis. Ain Shams med. J., 26(5-6): 601.

Ghanem, M.H., Guirgis, F.K., Khalil, S. & El-Sawy, M., 1973. Immunoglobulins in bilharzial hepatic fibrosis. The effect of antibiotics. Alex. med. J., 19(4) 258.

Ghanem, M.H., Issa, I.A., Wafy, A.R.A. & Hagras, M., 1972. The anti-inflammatory effect of cyclophosphamide in bilharzial hepatic fibrosis. Alex. med. J., 18: 2.

Hillyer, G.V., 1969. Immunoprecipitin in Schistosoma mansoni infection. Exper. Parasit., 25: 376.

Kagan, I.G. & Pellegrino, J., 1961. A critical review of immunological methods for the diagnosis of bilharziasis. Bull. Wld Hlth Org., 25: 611.

Lehman, J.S., Jr., Higashi, G.I., Bassily, S. & Farid, Z., 1972. Rheumatoid factors in Salmonella and Schistosoma infections. Trans. roy. Soc. trop. Med. Hyg., 66(1): 125.

Michaux, J.L., 1966. Les immunoglobulines des Bantous à l'état normal et pathologique. Ann. Soc. Belg. Med. trop., 46: 483.

Moore, D.E. & Warren, K.S., 1967. Hepatosplenic S. mansoni and japonica compared in mice each infected with one pair of worms. Trans. roy. Soc. trop. Med. Hyg., 61: 164.

Sadun, E.H. & Gore, R.W., 1970. S. mansoni and S. haematobium: Homocytotropic reagent-like antibodies in infections of man and experimental animals. Exp. Parasit., 28: 435.

Salah, M., 1962. The bilharzial liver. Alex. med. J., 8: 177.

Sherlock, S., 1970. Immunology of liver diseases. Amer. J. Med., 9(3): 693.

Trigger, D.R., Alp, M.H. & Wright, R., 1972. Bacterial and dietary antibodies in liver diseases. Lancet, 1: 60.

Von Lichtenberg, F., 1964. Studies on granuloma formation. III. Antigen sequestration and destruction in the schistosome pseudotubercle. Amer. J. Path., 45: 75.

Warren, K.S., Domingo, E.O. & Cowan, B.T., 1967. Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. Amer. J. Path., 51: 735.

A POSSIBLE CONTRIBUTING EFFECT OF ETHINYL OESTRADIOL ON THE PATHOGENESIS OF BILHARZIAL HEPATIC CIRRHOSIS AND SPLENOMEGALY

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Nicol et al. (1952) reported that oestrogen stimulated the reticuloendothelial system and caused mobilization of the splenic and liver macrophage cells into the circulation in massive numbers. Their accumulation in the secondary sex organs produced marked fibrosis in them.

El-Heneidy et al. (1966, 1968) demonstrated, in guinea pigs, that massive invasion of the different tissues by macrophages occurred mainly after 3 weeks of oral oestrogen intake. They showed that the early changes in the different organs were not due to local capillaritis or venulitis, but form a part of a general disease affecting the vascular endothelium which became hypertrophied, assuming a cuboidal or columnar type and forming more than one layer. Mononuclear cell invasion of the media and adventitia was apparent. These endothelial changes seem to favour cellular trapping of the circulating macrophages and other round cells with their subsequent migration to the tissue spaces. It also apparently leads to degeneration and fibrous tissue formation in different organs.

El-Ghazzawi et al. (1974) showed that orally given oestrogen caused hypertrophy of the germinal centres in the cortical nodules of guinea pig lymph nodes as well as initiation of a massive cellular infiltration in the paracortical area of

Turk. The infiltrating cells were mainly large pyroninophilic cells containing many ribonucleic acid granules in their cytoplasm ; they were proved by electron microscopy to be clusters or ribosomes or polysomes.

In addition to these pyroninophilic cells, there were many histiocytes, plasma cells and many lymphocytes. In other words, oestrogen initiated both an immediate as well as a cell mediated type of reaction, a finding which simulates that described by Oort & Turk (1965) for the immunological response of lymph nodes. It is difficult to explain the exact mechanism of the reaction. An interesting observation was that the cellular infiltration in the paracortical area was cyclic every 7 days. The mechanism of this periodicity in nodal reaction to oestrogen might be effected through other endocrine glands.

The aim of the present study was :

(1) To study the effect of orally given oestrogen on the mucopolysaccharides and lipids of the aortic wall in guinea pigs ;

(2) To study histopathologically, in patients undergoing the operation of splenectomy for bilharzial hepatosplenomegaly, biopsy material from the spleen and splenic lymph node and to examine the splenic artery for its mucopolysaccharide and lipid content.

Material and Methods

1. Guinea pigs

Twenty-five guinea pigs, all males, weighing 250-300 grams, were given oestrogen orally in the form of ethinyl oestradiol tablets 10 μg (Roussel-Paris) daily. They were divided into 5 groups of 5 animals each to study the effect of oestrogen on the mucopolysaccharides and on the lipid contents of the aortic wall with increasing duration of drug intake, as follows :

Group I : normal controls receiving no oestrogen

Group II : receiving the tablets for 2 weeks

Group III : receiving the tablets for 4 weeks

Group IV : receiving the tablets for 6 weeks

Group V : receiving the tablets for 8 weeks.

The animals in all groups were fed on a cholesterol free diet. Blood cholesterol levels were measured in all animals before oestrogen intake, and weekly thereafter.

The following methods were applied for demonstrating the presence of :

a) *Mucopolysaccharides*. Small pieces of the abdominal part of the aorta were taken and fixed in 10% neutral formal saline. Paraffin sections were prepared and stained with haematoxylin and eosin as well as with toluidine blue for metachromasia at pH 4.5 for permanent preparation as the colour fainted rapidly. This was done by using a 0.25% solution of toluidine blue made up in Michaelis veronal acetate hydrochloric acid buffer solution at pH 4.5 (Carlton, 1967). Other sections were stained with alcian blue both

at pH 2.2 or hyaluronic acid which was strongly stained at this pH (Zugibe, 1963) and at pH 1 for sulphated mucopolysaccharides.

b) *Lipids*. The osmium tetroxide alpha naphthylamine (OTAN) method was used to distinguish between unsaturated hydrophobic and hydrophilic lipids (Adams, 1959). Small pieces of the abdominal aorta fixed in calcium-formol solution were cut as frozen sections of 0.1 mm thickness. The free floating frozen sections were then treated with osmium tetroxide potassium chlorate solution for 18 hr (1 part 1% OsO_4 + 3 parts 1% $KClO_3$), and were then allowed to react with saturated aqueous alpha naphthylamine at 37°C for 10 min. Sections were finally washed with distilled water and mounted in Apathy's.

c) *Elastic laminae*. Gomor's aldehyde fuchsin method was used.

d) *Electron microscopic examination*. Very small fresh pieces of the aorta from the last group were fixed in chilled 6% glutaraldehyde at pH 7.4 for 2 hr followed by post fixation in chilled 1% osmium tetroxide saccharose buffered at pH 7.4. The tissues were dehydrated in graded alcohol and embedded in araldite at 50°C overnight in vacuum (Pease, 1964). Ultra-thin sections were stained with uranyl acetate and lead citrate.

2. Human patients

Ten male non-ascitic patients with bilharzial hepatic cirrhosis and splenomegaly, taken at random, were operated upon for splenectomy. The spleen and splenic lymph nodes were studied histopathologically in comparison with normal tissue. The splenic artery was investigated for mucopolysaccharides and lipids in the same way as described under 1.

Results

1. Guinea pigs

The mean for cholesterol in normal guinea pigs was 40 mg%. After the intake of ethinyl oestradiol the blood cholesterol started to rise. The mean, after the second week, was 70 mg%, at the end of the 4th week 165 mg%, after the 6th week 180 mg% and at the end of the 8th week it had reached 185 mg%. Therefore, oestrogen produced hypercholesterolemia.

a) *Mucopolysaccharides*. The tunica media of the aorta in the normal group of animals was weekly stained with alcian blue stain at pH 2.2 indicating a moderate amount of hyaluronic acid. Using toluidine blue stain at pH 4.5, faint metachromasia in the ground substance of the media was observed (Fig. 1), indicating a normal amount of acid mucopolysaccharides (AMPS) in the guinea pig's aorta.

After administration of oestrogen for 3 weeks, the inner zone of the media started to show an intense reaction for metachromasia (Fig. 2), as well as for alcianophilia. The metachromasia and alcianophilia were progressively increased with the prolonged intake of oestrogen in Groups III and IV until they reached a maximum reaction throughout the whole thickness of the media in Group V (Fig. 3).

b) *The lipids*. By the OTAN method, the phospholipids were detected in the medial elastic laminae of the aorta of normal guinea pigs (Fig. 4) as indicated by their faint orange-red staining. No cholesterol or free fatty acids were detected in this control group. The reaction for phospholipids was slightly increased in Group II, as indicated by the deep orange-brown colour of the phospholipids, especially in the inner zone of the media. It was noticed that the thickness of the wall as a whole was increased. The adventitia showed a few phagocytic cells, which stained black. An increased reaction was also observed in the elastic laminae together with accumulation of phospholipids in the intima, which was slightly thickened. No cholesterol esters were seen. With continued administration of oestrogen for 4 or more weeks in Groups III, IV and V, lipid laden phagocytes containing cholesterol esters, revealed by their black colour with OTAN, were seen in the subendothelial region of the intima (Fig. 5). Besides, the reaction for phospholipids in the media was intense and the adventitial phagocytic cells were increased. These changes were more pronounced with the prolonged intake of oestrogen. The blood cholesterol showed a rise with continuous administration and reached its maximum at the end of the test. In other words, ethinyl oestradiol produced hypercholesterolemia with a tendency towards atheromatous formation.

c) *The elastic laminae*. Oestrogen caused no pathological changes in the aortic elastic laminae.

d) *Electron microscopic observations*. The tunica intima was thickened. The endothelium was hypertrophied, with microvilli formation at its free surface. There were many histiocytes appearing in the suber 'othelium. The most striking finding was the presence of a group of modified muscle cells in the subendothelium, some of which showed empty vacuoles (Fig. 6). The tunica media revealed extensive vacuolization in the ground substance. These vacuoles were of different sizes; most of them were empty while others contained osmiophilic granules. Intracellular lamellated inclusions, called liposomes by Weller (1966), were seen in the field (Fig. 7).

2. Human patients

Splenic biopsy and lymph nodes. The spleen showed a thickened capsule and trabeculae (Fig. 8). Its white pulp showed well developed germinal centres containing many large pyroninophilic cells (Fig. 9) together with histiocytes. The follicular artery showed hyperplasia of its endothelium as well as a thickening of its media. The red pulp showed active congestion and some dilated sinusoids together with many large pyroninophilic cells, plasma cells, histiocytes (Fig. 10) and lymphocytes. The splenic nodes were hypertrophied with a mildly thickened capsule. The nodular lymphoid tissue, cortical and medullary, consisted mainly of large pyroninophilic cells and many histiocytes. The paracortical area showed similar cellular infiltration with some endothelial hypertrophy of the blood capillaries of loose lymphoid tissue (Fig. 11).
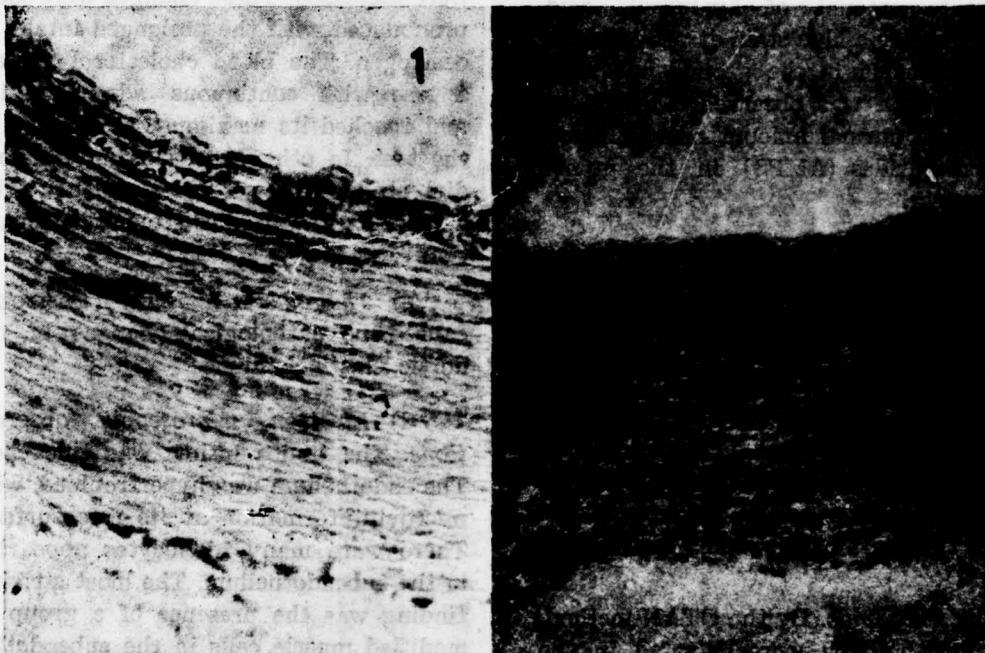

Fig. 1. Normal guinea pig aorta showing the normal metachromasia for acid mucopolysaccharides in the inner zone of the tunica media, while the outer zone is relatively negative. Stain, toluidine blue, pH 4.5 (x500).

Fig. 2. Guinea pig aorta after 3 weeks of oestrogen intake. The inner zone of the media started to show an increased reaction for metachromasia. (x500).
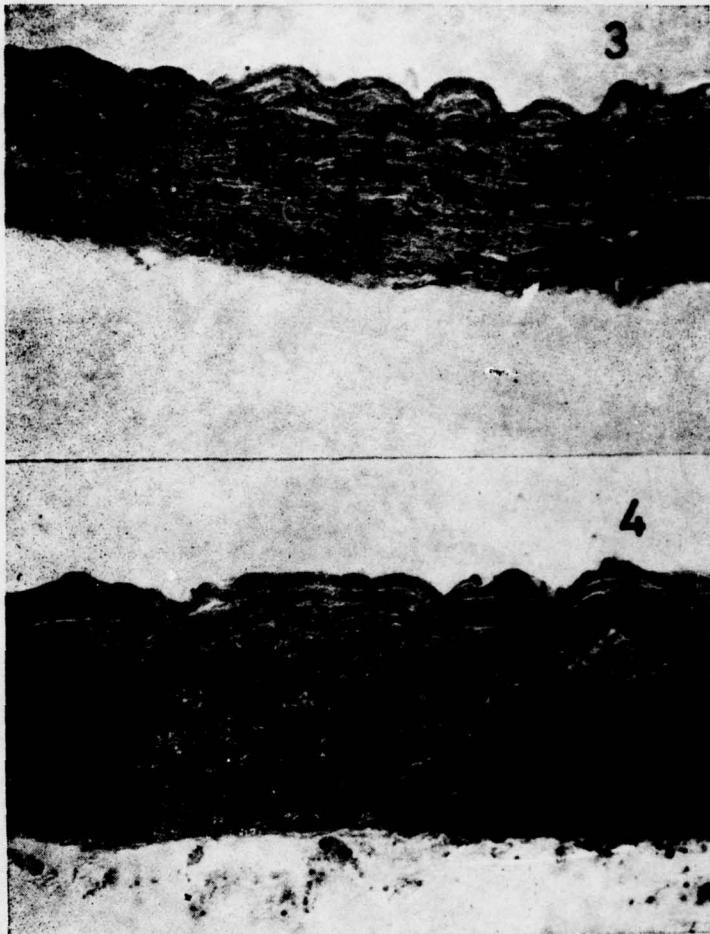
Fig. 3. Using the OTAN method (Adams, 1959), the phospholipids were detected in the tunica media of normal guinea pig aorta as well as in the elastic membrane, as indicated by their faint orange-red staining. No cholesterol or free fatty acids were detected (x320).

Fig. 4. After intake of oral oestrogen, the reaction for phospholipids was increased in the tunica media of the guinea pigs aortae. With continuous administration of oestrogen for 2 months, cholesterol esters, revealed by their black colour with OTAN, were observed in the macrophages (x320).
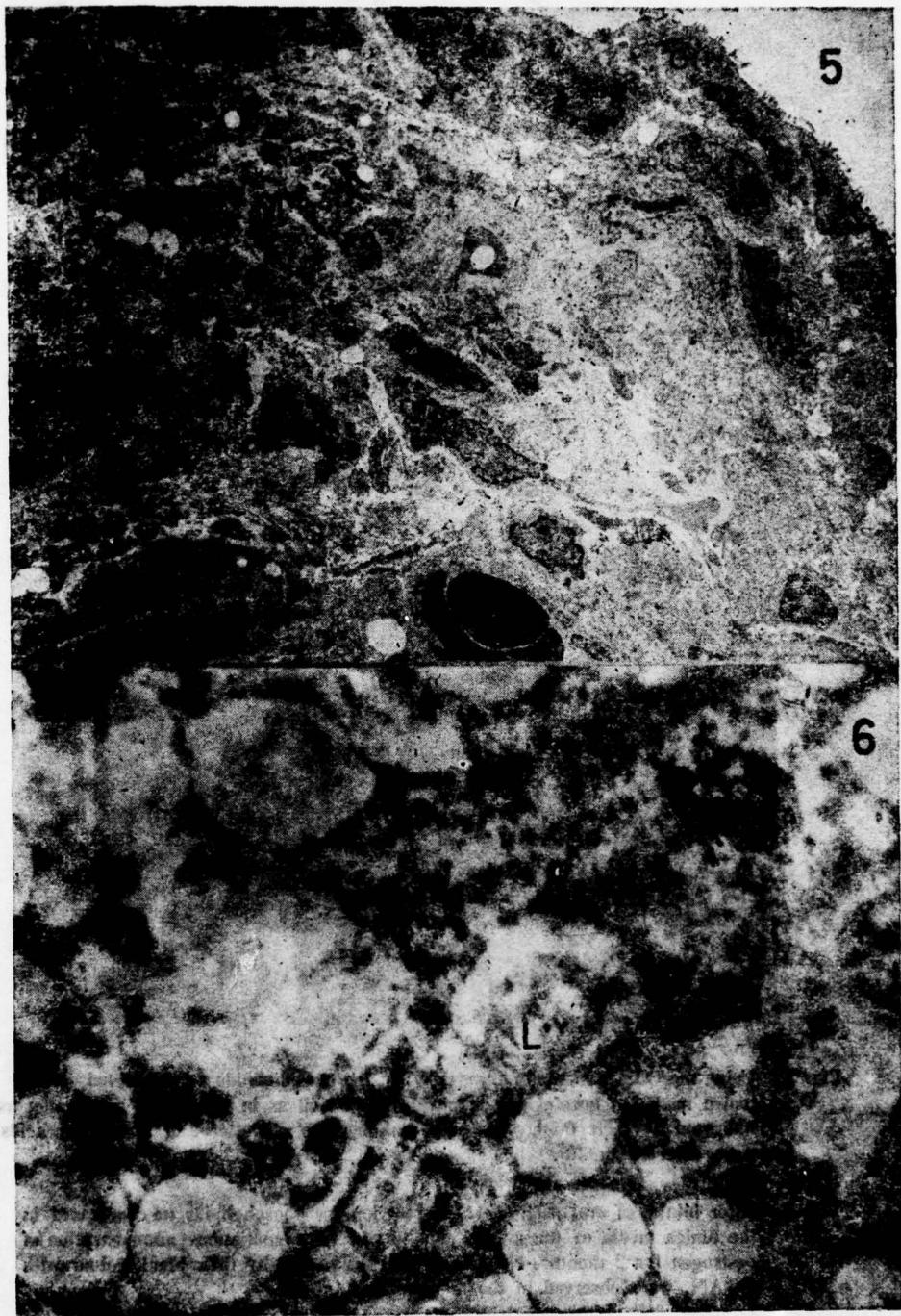
Fig. 5. Electron micrograph of aortic tunica intima from a guinea pig receiving oestrogen for 1 month, showing hypertrophied endothelial cells (en) with microvilli. The subendothelium shows many histiocytes as well as a group of modified myo-intimal cells (M) (x4000).

Fig. 6. Electron micrograph of the tunica media of the aorta from a guinea pig receiving oestrogen for 1 month, showing marked vacuolization in the ground substance. Some vacuoles are empty while others contain osmiophilic granules. Intracellular lamellated inclusion (L) is seen in the field (x20,000).
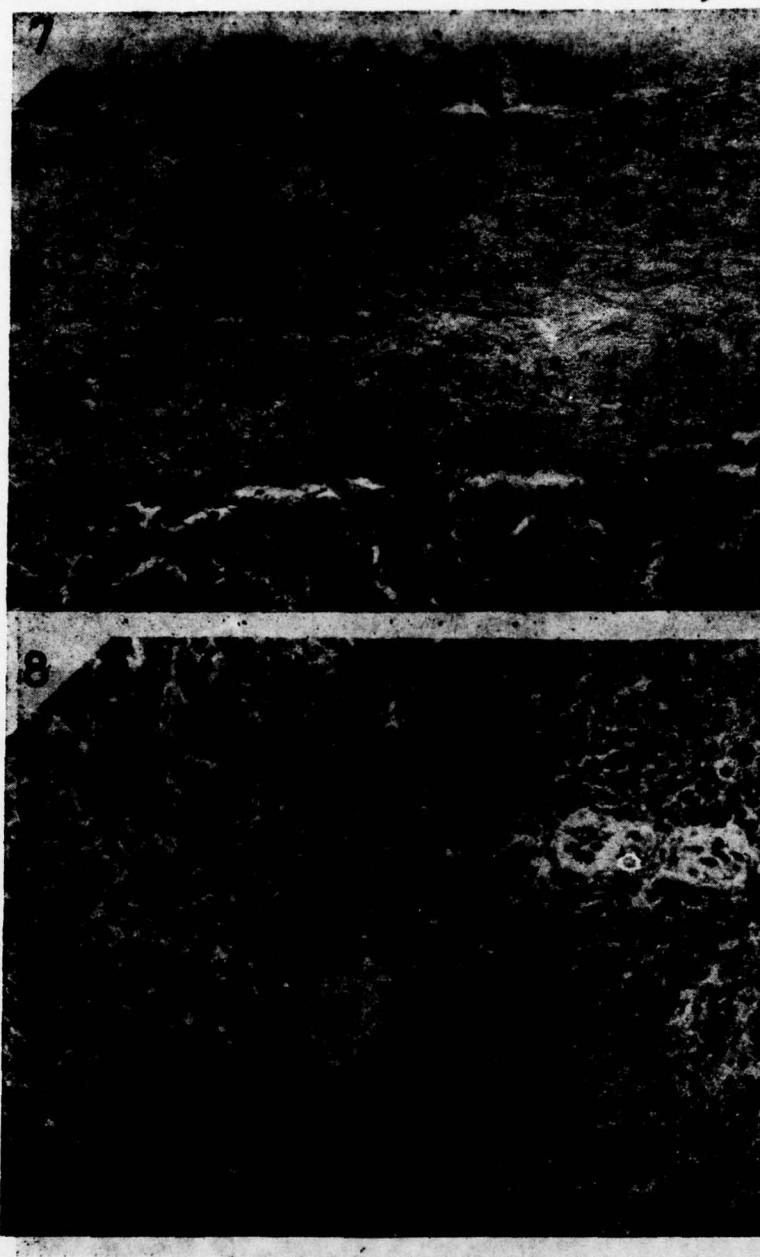
Fig. 7. The splenic capsule in hepatosplenic schistosomiasis shows marked thickening. Haematoxylin and eosin (x450).

Fig. 8. The white pulp of the spleen in hepatosplenic schistosomiasis showing well developed germinal centres containing many large pyroninophilic cells. The follicular artery shows hyperplasia of its endothelium as well as thickening of its media. Haematoxylin and eosin (x450).
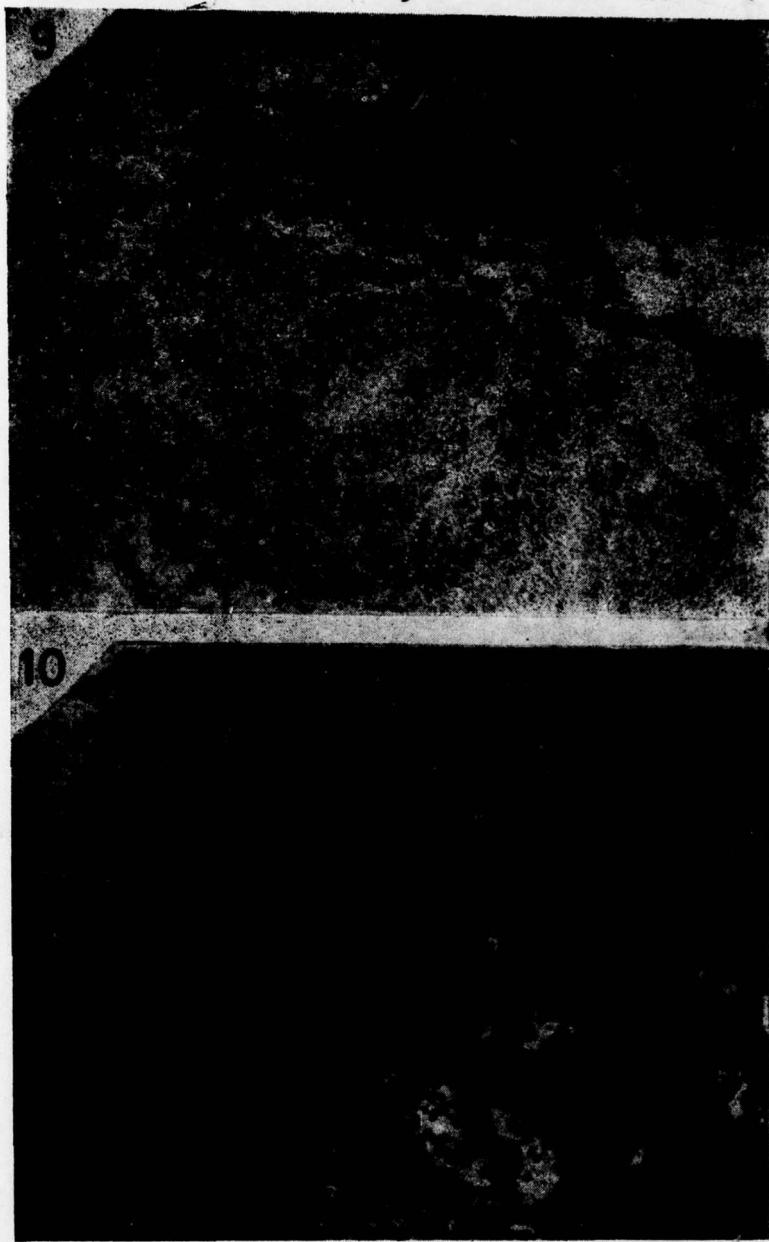
Fig. 9. Increased number and distribution of histiocytes in the splenic pulp of hepato-splenic schistosomiasis (x450).

Fig. 10. Splenic node showing mild capsular thickening. The nodular lymphoid tissue consists mainly of large pyroninophilic cells and many histiocytes. The paracortical area shows similar cellular infiltration (x450).
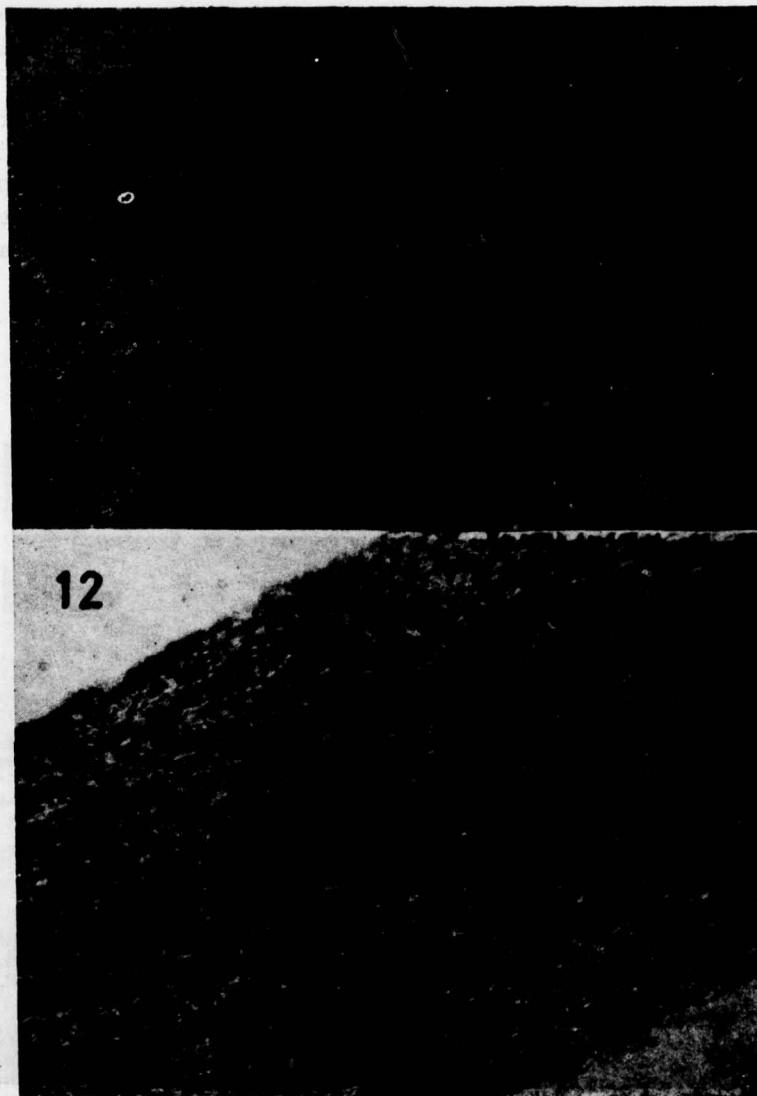
Fig. 11. Splenic artery from a normal human (control) showing a weak reaction to alcian blue (x500).

Fig. 12. Splenic artery from a case with hepatosplenic schistosomiasis with an intense reaction for metachromasia throughout the whole thickness of the tunica media, indicating increased acid mucopolysaccharide content. Stain, toluidine blue, pH 4.5 (x500).

Splenic artery

a) *Acid mucopolysaccharides (AMPS)*.
The normal human arteries reveal-
ed a weak reaction with alcian blue
and toluidine blue in the ground sub-
stance of the media (Fig. 12). In cases of
bilharziasis, the amount of AMPS was
greatly increased throughout the whole
media, as evidenced by the strong reac-
tion with both alcian blue and toluidine
blue.

b) *Lipids*. The control human arteries
showed a moderate reaction in the tunica
media with the OTAN method. The in-
ternal elastic lamina also revealed a posi-
tive reaction. No black staining hydro-
phobic lipids were detected.

In the splenic artery of bilharzial
patients both the phospholipid content of
the media as well as the sphingomyelin
content of the vessel wall were increased
as compared to the normal arteries (Fig.
13).
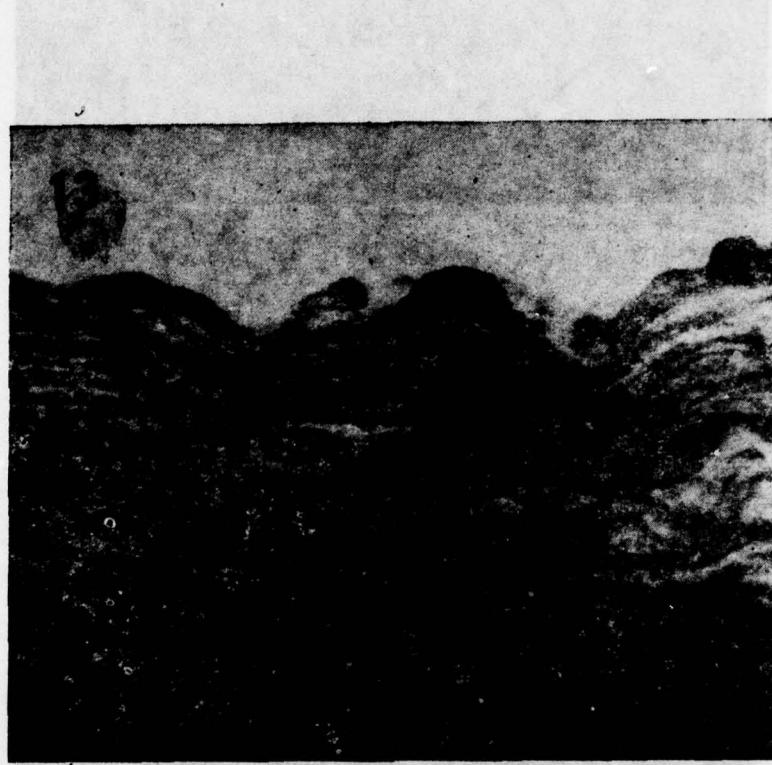
Fig. 13. The splenic artery from a case with hepatosplenic schistosomiasis, using the OTAN method. Phospholipids were detected in the tunica media and elastic membrane. Cholesterol esters, revealed by their black colour with OTAN, were observed in the macrophages (x500).

Discussion

Oestrogen has a strikingly stimulating effect on the reticuloendothelial system with macrophage cell mobilisation and cellular trapping. This is followed by infiltration and fibrosis in different organs (Nicol et al., 1952 ; El-Heneidy et al., 1966, 1968). El-Heneidy et al. (1973) studied the effect of local splenic subcapsular implantation of ethinyl oestradiol depot on the reticuloendothelial system in the portal tract in 4 mongrel dogs. The drug was implanted 4 times at intervals of $1\frac{1}{2}$ months. At the same time sterilised starch was implanted in the same way in 2 mongrel dogs which were used as controls. The main findings were as follows :

In animals treated with sterile starch, the spleen was within normal size ; it showed slight thickening of the capsule and trabeculae, but no other changes from normal. The draining lymph nodes of the spleen were normal as regards size and histological structure.

In animals treated with ethinyl oestradiol, the spleen had nearly doubled its size and was coated with a tough capsule. In cut section, its trabeculae were found well developed and had maintained their original structure. The draining lymph nodes were hypertrophied, but the liver showed no gross changes. However, the pancreas showed moderate thickening of its capsule. Microscopically, the spleen showed well developed germinal centres which consisted mainly of large pyroninophilic cells (lymphoblasts) and histiocytes. The follicular artery showed hyperplasia of its endothelium as well as thickening of the media. The red pulp of the spleen showed active congestion and some dilated blood sinusoids. There were many large pyroninophilic cells together with plasma cells, numerous histiocytes and lymphocytes. The draining lymph nodes showed a partial loss of their general architecture and their capsules were mildly thickened. The nodular lymphoid tissue, cortical and medullary, consisted mainly of large pyroninophilic cells, many histiocytes (mainly at the periphery of the cortical nodules) with well developed germinal centres. The loose lymphoid tissue of the paracortical area demonstrated a marked cellular infiltration consisting of numerous histiocytes, plasma cells, lymphocytes and large pyroninophilic cells. Histiocytes, demonstrated by the vital dye, trypan blue, showed that their number was very much increased in the loose lymphoid tissue.

The same changes occurred in the cortical nodules both at their periphery and in their centres. No trypan blue stain was demonstrated in the spleen.

The lymph sinuses were dilated and contained many histiocytes showing the dye in their cytoplasm, the littoral cells lining the sinuses were hypertrophied and took the dye (sinus histiocytosis). The increased number of histiocytes in the draining lymph nodes is probably due to their activation from the continued release of ethinyl oestradiol from under the capsule of the spleen. By this study it was demonstrated that local implantation of long acting ethinyl oestradiol under the capsule of the spleen in mongrel dogs provoked striking changes in that organ as well as in its draining lymph nodes. The changes included reticuloendothelial hyperplasia as well as cellular infiltration similar to that found in guinea pigs receiving ethinyl oestradiol orally.

In the second part of this study, the lymph node changes (splenic nodes in bilharzial hepatosplenomegaly) were so similar to those seen in the splenic nodes

of mongrel dogs subjected to subcapsular implantation of ethinyl oestradiol that it is difficult to differentiate between the two under the microscope. In the part of this study that deals with guinea pigs, it was demonstrated that ethinyl oestradiol caused an increase in the amount of acid mucopolysaccharides and hyaluronic acid of the ground substance of the aortic wall.

The drug also increased the phospholipid content of the vessel wall and caused accumulation of cholesterol esters in the intima. In bilharzial patients, the splenic artery showed an increase in acid mucopolysaccharides and phospholipids. These changes were very similar to those met with in the aortae of guinea pigs receiving ethinyl oestradiol orally for 2 months. From these studies the following hypothesis is advanced :

Feminisation is not uncommon in bilharzial hepatic cirrhosis and splenomegaly, and hyperoestronaemia is a common finding.

The reticuloendothelial cells represent the most primitive type of mesodermal cells that are still pluripotent. Triggered by the proper stimulus, these apparently dormant cells are activated and can repeat the evolution of their ancestral forms. The cells hypertrophy, proliferate and/or migrate to develop into mature cells. They also invade other tissues, proving that even the final atrophy and fibrosis of the invaded tissues is another face of an active process of this still unexplored type of cells.

The largest aggregation of the reticuloendothelial cells of the body occurs in the liver and the spleen. It is possible that an increase in oestrogen level in patients with hepatic cirrhosis may be responsible, at least in part, for the hyperplasia of the reticuloendothelial system in the portal area met with in such cases. It may thus contribute to an increase in the number of the reticuloendothelial cells in the liver and the spleen, and increases further the hepatomegaly and splenomegaly.

LITERATURE CITED

Adam, S.C.W.M., 1959. A histochemical method for the simultaneous demonstration of normal and degenerating myelin. J. Path. Bact., 77: 648-650.

Carlton, S., 1967. «Histological Technique». Fourth edition. Revised and rewritten by R.A.B. Drury and E.A. Wallington. Oxford Univ. Press, New York, Toronto. p. 214-215.

El-Ghazzawi, Ebtihag F., Sorour, S., El-Gendi, M.A., Malaty, H.A. & El-Heneidy, A.E., 1974. The histological changes in lymph nodes in response to oral oestrogen. Bull. Alex. Fac. Med. (in press).

El-Heneidy, A.R., El-Gendi, M.A. & El-Ghazzawi, Ebtihag F., 1973. Experimental splenomegaly by subcapsular implantation of oestrogen. Alex. med. J., 19(4).

El-Heneidy, A.R., Helmy, I.D. & Michael, M.A., 1966. Experimental diffuse interstitial fibrosis of the lung and capillary cellular trapping. Alex. med. J., 12: 275.

El-Heneidy, A.R., Helmy, I.D. & Michael, M.A., 1968. Experimental evolution of rheumatoid disease and capillary cellular trapping. Alex. med. J., 14: 143.

Nicol, T., Helmy, I.D. & Abou-Zikry, A., 1952. A histological experimentation for beneficial action of endocrine therapy in carcinoma of the prostate. Brit. J. Surg., 40: 166.

Oort, J. & Turk, J.L., 1965. A histological and autoradiographic study of lymph nodes during the development of contact sensitivity in the guinea pig. Brit. J. exp. Path., 46: 146.

Pease, B.C., 1964. «Histological Technique for Electron Microscopy». Second edition. Academic Press, New York.

Weller, R.O., 1966. The ultrastructure of intracellular lipid accumulation in atheroma. J. Atherosclerosis Res., 6: 184-189.

Zugibe, F.T., 1963. Mucopolysaccharides of the arterial wall. J. Histochem. Cytochem., 11: 35-39.

DISTINCTIVE ADJUVANTICITY IN SALINE OF SYNTHETIC
ANALOGS OF MYCOBACTERIAL WATER SOLUBLE COMPONENTS

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There exist several immunological aspects in the relationship between the host and the schistosomal parasite. Promising experimental results have been obtained with sera of infected patients or of hyperimmunized animals which would allow eventually the use of a vaccine. Such considerations are strongly in favor of the utilization of adjuvants, since schistosomal antigens are generally considered as being weak immunogens.

The extraordinary effectiveness of Freund's complete adjuvant (FCA) in amplifying the immune responses has been extensively documented during the past quarter century, but its utilization other than in experimental procedures has been restricted by the toxic reactivity of mycobacteria and the non-metabolizable oil component.

The chemistry of adjuvant fractions of mycobacteria has been investigated for many years, but it is only recently that the relevant structures have been identified. It has been established that water soluble fractions can substitute for mycobacterial cells in FCA but are devoid of the «side effects» elicited by the administration of heat-killed organisms (Adam et al., 1972 ; Hiu, 1972 ; Migliore-Samour & Jolles, 1972 ; Stewart-Tull et al., 1975). One such component, referred to as water soluble adjuvant (WSA) was extracted by Adam et al. (1972) from *Mycobacterium smegmatis* and proved to be an arabinogalactan linked to a peptidoglycan. Subsequently neutral sugars were removed from the peptidoglycan moiety (Adam et al., 1974a) and monomeric peptidoglycans extracted from *M. smegmatis* and *Escherichia coli* also turned out to be active adjuvants (Adam et al., 1974b ; Fleck et al., 1974). Most recently, it was demonstrated that synthetic N-acetyl-muramyl-L-alanyl D-isoglutamine (MDP$_1$) had the minimal structure required for adjuvant activity (Ellouz et al., 1974 ; Kotani et al., 1975). In the latter studies, the adjuvant activity was demonstrated by immunization of guinea pigs with ovalbumin in a water-in-oil (Bayol F, Arlacel) emulsion : precipitating antibodies were increased and delayed hypersensitivity was induced. Although WSA administered with antigen in saline has also been shown to increase the immune response both *in vivo* (Parant & Chedid, 1974) and *in vitro* (Modolell et al., 1974),

N-ACETYL-MURAMYL-L-ALANYL-D-GLUTAMIQUE   N-ACETYL-MURAMYL-L-ALANYL-D-ISOGLUTAMINE (MDP₁)
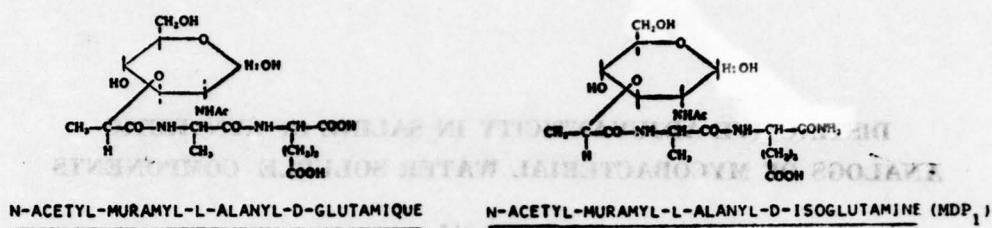
Fig. 1. Synthetic analogs

this effect was slight compared to that obtained with the same material administered in Freund's incomplete adjuvant (FIA).

In contrast to mycobacterial adjuvant preparations that function only in the form of water-in-oil emulsion, synthetic N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP₁) and N-acetyl-muramyl-L-alanyl-D-isoglutamic acid (Fig. 1) augment the humoral immune responses of mice equally well as aqueous solutions (see Table 1).

Whereas N-acetyl-muramyl-L-alanyl-D-isoglutamic acid administered to guinea pigs in water-in-oil emulsion has no effect, N-acetyl-muramyl-L-alanyl-D-isoglutamine induces delayed hypersensitivity to ovalbumin and ABA-tyrosine and increases the level of antibody against ovalbumin. Under these conditions challenge with the synthetic adjuvants themselves evokes no skin responses. Moreover, Freund's complete adjuvant sensi-

tizes guinea pigs to tuberculin and to native mycobacterial water soluble adjuvant but not to the synthetic analogs (Audibert et al., 1976) (see Tables 2 and 3).

It was also observed that MDP₁ is adjuvant active by several routes and even by the oral route (see Table 4). In view of studying the relation between chemical structure and biological activity, several synthetic analogs were tested. The immune response could be modulated according to chemical modifications and two synthetic analogs were shown to inhibit the immune response (Chedid et al., 1976) (see Table 5).

Non toxic synthetic adjuvants, highly effective in augmenting antibody response without any other suplementation, would offer important advantages for human public health immunization. These compounds would also provide a distinctive means for definitive studies of the molecular biology and genetics of adjuvant action.

TABLE 1. Adjuvant Action of Mur-NAc-L-Ala-D-iso-Gln and Mur-NAc-L-Ala-D-Glu on the Humoral Response of Mice to Bovine Serum Albumin (BSA) Injected in Saline

Immunization	Primary response						Secondary response	
	Day 14		Day 28		Day 34		Day 36	
	PA+	ABC+	PA	ABC	PA	ABC	PA	ABC
Antigen (controls)	<3	<20	3	<20	<3	20	12 ± 15	<20
Antigen + LPS++ 30 µg	<3	<20	6	<20	100	120	715 ± 225**	530
Antigen + LPS 100 µg	<3	<20	6	<20	50	110	1,300 ± 510**	1,600
Antigen + Mur-Nc-L-Ala-D-iso-Gln 30 µg	3	<20	25	75	200	210	800 ± 350**	400
Antigen + Mur-NAc-L-Ala-D-iso-Gln 300 µg	<3	<20	25	50	200	230	975 ± 550**	550

Immunization	Day 14		Day 28		Day 34		Day 36	
	PA+	ABC+	PA	ABC	PA	ABC	PA	ABC
Antigen (controls)	<3	<20	<3	<20	3	<20	3 ± 6	<20
Antigen + WSA 300 µg.........	<3	<20	<3	<20	6	<20	6 ± 4	65
Antigen + Mur-NAc-L-Ala-D-iso-Gln 100 µg	<3	<20	25	82	400	270	2,150 ± 1,900**	520
Antigen + Mur-NAc-L-Ala-D-Glu 100 µg	3	<20	50	80	400	320	2,130 + 900**	630

+ PA: passive hemagglutination   ABC: antigen binding capacity

++ Lipopolysaccharides of gram-negative bacteria

At days 4, 28 and 34, titers were evaluated on pooled sera for each group. At day 36, sera were collected separately.

** Significance was calculated by student's test: ** = $p < 0.01$.

TABLE 2. Adjuvant Activity of Synthetic Analogues on Immune Response of Guinea Pigs to Ovalbumin in FIA

Immunization	Skin reactions* to 5 µg ovalbumin mm diameter	Antibodies against ovalbumin	
		Precipitation µg/ml	Agglutination Titers
Experiment 1			
Antigen + FIA (controls)	0,0,0,0,0	700	3,200
Antigen + FCA	11,14,12,15	3,200	20,000
Antigen + FIA + Mur-NAc-L-Ala-D-iso-Gln 10 µg	10,5,8,2,4	3,000	15,000
Antigen + FIA + Mur-NAc-L-Ala-D-iso-Gln 50 µg	12,15,15,14,16	5,000	18,000
Experiment 2			
Antigen + FIA (controls)	0,0,0,0,0,0	500	1,000
Antigen + FCA	10,8,10,12,11	3,200	2,400
Antigen + FIA + Mur-NAc-L-Ala-D-iso-Gln 10 µg	8,8,7,8,10,12	5,000	4,000
Antigen + FIA + Mur-NAc-L-Ala-D-Glu 50 µg	0, 0, 5, 6	500	900

* Reactions 48 hours after ovalbumin challenge on day 18.

FIA = Freund's incomplete adjuvant

FcA = Freund's complete adjuvant

TABLE 3. Absence of delayed hypersensitivity to tuberculin and Mur-NAc-L-Ala-D-iso-Gln or D-Gln in guinea pigs sensitized by the synthetic analogues with FIA

Immunization	Skin reactions (mm diameter)*				
	Purified tuberculin 50 IU	Old tuberculin 50 IU	WSA 5 µg	Mur-NAc-L-Ala-D-Glu 5 µg	Mur-NAc-L-Ala-D-iso-Gln 5 µg
FCA	10,7,10,7,10,7	14,10,13,12,13,5	6,7,0,6,3,5	0,0,0,0,0,0	0,0,0,0,0,0
FIA + Mur-NAc-L-Ala-D-iso-Gln 50 µg	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0
FIA + Mur-NAc-L-Ala-D-Glu 50 µg	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0

* Reactions 48 hr. after challenge on day 18.

TABLE 4. Adjuvanticity of Mur-NAc-L-Ala-D-iso-Gln and Mur-NAc-L-Ala-D-Glu on the humoral response of mice to bovine serum albumin adiimlnistered by the same or by different routes.

Immunization[+]	Adj. dose (µg)	Adj.[++] route	Ag.[++] route	Day 14	Day 28	Day 34	Day 36
Antigen (controls)	—	—	SC	<3	<3	<3	6
Antigen (controls)	—	—	IV	<3	<3	50	200
Antigen + Mur-NAc-L-Ala-D-iso-Gln	0.1	SC	SC	25	50	400	1,620**
Antigen + Mur-NAc-L-Ala-D-iso-Gln	0.1	IV	SC	12	12	200	1,010**
Antigen + Mur-NAc-L-Ala-D-iso-Gln	0.1	SC	IV	50	50	100	790*
Antigen + Mur-NAc-L-Ala-D-iso-Gln	2	PO	SC	6	12	200	1,350**
Antigen + Mur-NAc-L-Ala-D-iso-Gln	1	PO	SC	6	6	200	850**
Antigen + Mur-NAc-L-Ala-D-iso-Gln	0.1	PO	SC	<3	<3	25	50
Antigen + Mur-NAc-L-Ala-D-Glu	1	PO	SC	3	6	100	140

+ 8 mice per group received 0.5 mg of antigen and adjuvant when noted. At day 30, every mouse was boosted subcutaneously with 100 µg of antigen.

++ SC = subcutaneous   IV = intravenous   PO = per os

At day 14, 28 and 34, titers were evaluated by passive hemagglutination on pooled sera for each group. At day 36, sera were collected separately. Significance was calculated by Student's test * = $p < 0.05$; ** = $p < 0.01$ when comparing experimental groups with their respective controls.

TABLE 5. Comparison between the activity of different analogs of Mur-NAc-L-Ala-D-iso-Gln on the humoral response of mice to high dosages of bovine serum albumin (BSA) injected in saline.

Immunization*	Day 14	Day 28	Day 34	Day 36
Antigen (controls)	<3	<3	<3	<3
Antigen + LPS	<3	6	50	1,310**
Antigen + Mur-NAc-L-Ala-D-iso-Gln	6	6	200	1,620**
Antigen + Mur-NAc-L-Ala-D-Glu	3	12	400	1,315**
Antigen + Mur-NAc-L-Ser-D-iso-Gln	12	25	1,600	1,600**
Antigen + Mur-NAc-Gly-D-iso-Gln	6	3	200	200
Antigen + Mur-NAc-L-Ala-D-Glu (OMe) NH_2	12	12	100	800**
Antigen + Mur-NAc-L-Ala-D-Glu (OMe) OMe	50	50	400	1,800**
Antigen + Mur-NAc-L-Ala-D-Glu-α-$NHCH_3$	6	12	1,600	1,600**
Antigen + Mur-NAc-L-Ala-D-Glu (NH_2) NH_2	<3	<3	<3	<3
Antigen + L-Ala-D-iso-Gln	<3	<3	<3	<3
Antigen + Mur-NAc-D-Ala-D-iso-Gln	<3	<3	3	3
Antigen + Mur-NAc-L-Ala-L-Glu	3	3	100	50
Antigen + Mur-NAc-L-Ala-L-iso-Gln	3	6	50	25
Antigen + WSA	<3	<3	<3	6

* 8 mice per group received subcutaneously, at day 1, 0.5 mg of BSA with or without 0.1 mg of adjuvant and, at day 30, a recall of 0.1 mg of antigen.

At day 14, 28 and 34, titers were evaluated by passive hemagglutination on pooled sera for each group. At day 36, sera were collected separately. Significance was calculated by Student's test.

(** $p < 0.01$).

LITERATURE CITED

Adam, A., Amar, C., Ciorbaru, R., Lederer, E., Petit, J.F. & Vilkas, E., 1974a. Activité adjuvante des peptidoglycanes de mycobactéries. C.R. Acad. Sci. Paris, ser. D, 278: 799-801.

Adam, A., Ciorbaru, R., Ellouz, F., Petit, J.F. & Lederer, E., 1974b. Adjuvant activity of monomeric bacterial cell wall peptidoglycans. Biochem. Biophys. Res. Commun., 56: 561-567.

Adam, A., Ciorbaru, R., Petit, J.F. & Lederer, E., 1972. Isolation and properties of a macromolecular, water soluble, immuno-adjuvant fraction from the cell wall of Mycobacterium smegmatis. Proc. natl. Acad. Sci. USA, 69: 851-854

Audibert, F., Chedid, L., Lefrancier, P. & Choay, J., 1976. Distinctive adjuvanticity of synthetic analogs of mycobacterial water soluble components. Cellular Immunol., 21: 243-249.

Chedid, L., Audibert, F., Lefrancier, P., Choay, J. & Lederer, E., 1976. Modulation of the immune response by a synthetic adjuvant and analogs. Proc. natl. Acad. Sci. USA, 73: 2472-2475.

Ellouz, F., Adam, A., Ciorbaru, R. & Lederer, E., 1974. Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. Biochem. Biophys. Res. Commun., 59: 1317-1325.

Fleck, J., Mock, M., Tytgat, F., Nauciel, C. & Minck, R., 1974. Adjuvant activity in delayed hypersensitivity of the peptidic part of bacterial peptidoglycans. Nature, 250: 517-518.

Hiu, I.J., 1972. Water soluble and lipid-free fraction from BCG with adjuvant and antitumor activity. Nature New Biol., 238: 241-242.

Kotani, S., Watanabe, Y., Kinoshita, F., Shimono, T., Morizaki, I., Shiba, T., Kusumoto, S., Tarumi, Y. & Ikenaka, K., 1975. Immunoadjuvant activities of synthetic N-acetyl muramyl-peptides or aminoacids. Biken J., 18: 105-111.

Migliore-Samour, D. & Jolles, P., 1972. A hydrosoluble, adjuvant-active mycobacterial «poly-saccharide-peptidoglycan». Preparation by a simple extraction technique of the bacterial cells (strain Peurois). FEBS Letters, 25: 301-304.

Modolell, M., Luckenbach, G.A., Parant, M. & Munder, P.G., 1974. The adjuvant activity of a mycobacterial water soluble adjuvant (WSA) in vitro. J. Immunol., 113: 395-403.

Parant, M. & Chedid, L., 1974. Biological properties of a non toxic water soluble immuno-adjuvant from mycobacterial cells. In: Mathe, G. & Weiner, R. (Eds.), Recent results in cancer research, 47: 190-195. Springer Verlag.

Stewart-Tull, D.E.S., Shimono, Y., Kotani, S., Kato, M., Ogawa, Y., Yamamura, Y., Koga, T. & Pearson, C.M., 1975. Adjuvant activity of a non-toxic, water soluble glycopeptide present in large quantities in the culture filtrate of Mycobacterium tuberculosis strain OT. Immunology, 29: 1-15.

ACQUIRED RESISTANCE TO *SCHISTOSOMA HAEMATOBIUM* IN THE BABOON (*PAPIO ANUBIS*) AFTER IMMUNISATION WITH ADULT WORMS

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ABSTRACT

Observations by Webbe and James (1973) and James et al. (1974) showed that baboons develop a strong acquired resistance to **Schistosoma haematobium** after prolonged periods (70-100 weeks) of immunisation but that the immunological protection conferred over short time intervals (27 weeks) was weak.

The present studies were undertaken to establish whether adult worms could stimulate an immune response. Fifty worm pairs were transferred from a baboon into the mesenteric vessels of three previously uninfected baboons. Three other baboons received 80-100 worm pairs each, obtained from rodents by perfusion. These six baboons were challenged in pairs, together with clean baboons, with 7,000 **S. haematobium** cercariae. The time interval between transplant and challenge varied between 35 and 55 weeks.

None of the baboons showed a strong acquired resistance, if the criteria for this are taken to be a reduction in expected egg excretion, adult worm recovery and tissue egg counts. No difference in the level of immunity was apparent in the baboons harbouring baboon donor worms compared with those having worms of rodent origin. No measurable increase in immunity occurred as the time interval between transfer and challenge was increased. There was, however, a considerable reduction in the pathology seen in immunised baboons compared with the challenge controls. The observed moderation of granuloma formation around eggs in the tissues was also observed in the short-term immunisation experiment (James et al., 1974). It is therefore considered that expression of a degree of acquired immunity should be based not only on egg excretion and worm burdens but also on the change in host reaction to the presence of ova in the tissues.

LITERATURE CITED

James, C., Webbe, G. & Nelson, G.S., 1974. Acquired resistance to Schistosoma haematobium in the baboon (Papio anubis). Proceedings of the Third International Congress of Parasitology, Munich, 25-31 August 1974 Vol. 2, p. 833.

Webbe, G. & James, C., 1973. Acquired resistance to Schistosoma haematobium in the baboon (Papio anubis). Transactions of the Royal Society of Tropical Medicine and Hygiene, 67: 151-152.

STUDIES ON THE MECHANISM OF ACQUIRED IMMUNITY
TO *SCHISTOSOMA MANSONI**

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ABSTRACT

Several recent studies in laboratory animals have indicated that acquired immunity to Schistosoma mansoni infection acts primarily against the migrating schistosomulum during the first few days after infection. Experiments are being carried out in this laboratory to characterize the various factors which play a role in this process. Attempts have been made to abrogate immunity by injecting immunized animals with drugs known to be effective in destroying specific types of leukocytes. These have included silica particles, 48/80 compound, and nitrogen mustard. In addition, various types of leukocytes have been tested in the presence and absence of immune serum for effects on schistosomula in vitro. Finally, the mechanism by which schistosomula become rapidly protected against the effects of immune serum and cells is being investigated.

Briefly, these studies indicate that both antibody and leukocytes, including polymorphonuclear leukocytes, are probably required for protective immunity to be expressed. The loss of schistosomulum susceptibility to in vitro destruction by antiserum and leukocytes appears to depend on active metabolic processes of the parasite. Drugs known to interfere with synthetic and metabolic processes, including uabain, reversibly block the development of this protective mechanism. The finding that schistosomula can become protected in defined culture media, in the absence of host macromolecules, indicates that protection may be independent of the surface adsorption of host antigens. The results obtained thus far suggest that a process involving either loss or rapid turnover of surface target antigens is involved.

TOWARDS THE DEVELOPMENT OF A LIVE VACCINE FOR SCHISTOSOMIASIS

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ABSTRACT

Control of human and domestic animal schistosomiasis would be greatly facilitated by the availability of effective vaccines. So far, the only experimental active immunisation procedures which have produced a good degree of protection have been those incorporating living schistosomes, and in our laboratory we are concentrating on this approach.

We have found that irradiated schistosomula of Schistosoma mattheei and S. mansoni can (a) be readily produced in large numbers without using passage through membranes, (b) are immunogenic in sheep and mice following intramuscular injection and (c) may be cryopreservable. The protection produced is quite long-lived: there was no decrease in protection at 30 weeks after a single immunisation of mice with 3 or 6 Kr irradiated S. mansoni cercariae compared with challenge at 8 weeks. Our collaborator Dr.

Mansour, of the Department of Veterinary Pathology, University of Khartoum, has obtained similar results using S. bovis in cattle in the Sudan: good protection was provided by a single exposure to irradiated cercariae and the immunity was long-lived. In fact, a greater worm reduction was obtained when challenge was at 24 weeks than at 8 weeks.

Field studies are now under way in the Sudan as a preliminary to carrying out the first field trials of an S. bovis irradiated vaccine in 1-2 years' time. Successful vaccination of domestic animals would not only be of great economic benefit but would also pave the way to the introduction of irradiated vaccines against human schistosomiasis. As a preliminary towards developing a human vaccine we are now attempting to immunise baboons with intramuscularly-injected irradiated schistosomules of S. mansoni.

USE OF SOLUBLE EGG ANTIGEN (SEA) IN THE IMMUNODIAGNOSIS OF HUMAN SCHISTOSOMIASIS

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ABSTRACT

Soluble egg antigen (SEA) prepared from Schistosoma mansoni and S. haematobium was used in the indirect hemagglutination test (IHA) and in a micro-system of the enzyme-linked immunosorbent assay (ELISA). The results are compared with those of the indirect immunofluorescence antibody test (IFAT) using frozen sections of adult S. mansoni as antigen.

To guarantee a high specificity of the methods, sera from patients with other helminth infections were tested for calibration of the minimal positive titer. For Swiss patients with S. mansoni infections, sensitivity for IFAT was 81%, for IHA with S. mansoni SEA 79%. The reactivity of these sera in ELISA was very low as in IHA with heterologous egg antigen.

Swiss patients infected with S. haematobium reacted to a lesser extent, i.e. only 60% in IFAT and 56% in IHA using S. mansoni SEA. A similar sensitivity was found in ELISA. The sensitivities of the methods used were also rather low for 28 sera of local patients heavily infected with S. haematobium from Madagascar; it was 61% in IFAT, 57% with heterologous and 68% with homologous SEA in ELISA.

In conclusion we can say that SEA is an antigen of interest for immunodiagnostic purposes. Fractionation of the crude SEA (isoelectric focusing showed over 30 proteins in the pH-range of 3.5 to 9.5) would eventually allow to set the specific titer at a lower level and thereby obtain a better sensitivity.

RAPID DETECTION OF SCHISTOSOMES IN BIOLOGICAL FLUID USING ACRIDINE ORANGE FLUORESCENCE

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Acridine orange has been used for a number of years as a diagnostic stain for D.N.A. and R.N.A., and also in the demonstration of malarial parasites in blood smears (WHO, 1969).

A very rapid technique was developed for staining parasites and bacteria in which a thin smear of the biological fluid was applied to a slide and air dried (Lewin, 1974). The preparations were fixed in 100% methanol for one minute, air dried and stained with 0.01% acridine orange (basic orange 14, B 199 AX 305, Matheson, Coleman & Bell Co.) for 30 seconds. The stain was then washed off with tap water and air dried. A drop of distilled water was then applied to the smear on the slides and covered over by a coverslip. These preparations were examined by reflected illumination under a Zeiss fluorescent microscope with an HBO-200 lamp excitor filter BG-12 and barrier filter 53-54.

Fluorescence could be detected during screening of biological smears at low magnification and the characteristic morphology of the micro-organisms confirmed at higher magnification (D.N.A. component staining greenish yellow and R.N.A. component staining red).

Schistosome ova were well demonstrated in urine obtained from a patient with schistosomiasis.

This technique may have wide use and warrant further investigations because the stain technique is simple, the reagents are cheap and parasites such as schistosomes are easily identifiable. Conventional stains made later on the same preparations are good.

LITERATURE CITED

Lewin, P.K., 1974. Rapid detection of bacteria and parasites using acridine orange fluorescence. International Research Communication System (IRCS) 2: 1599.

World Health Organization, 1969. Techn. Rep. Ser. No. 422: 70.

DELAYED HYPERSENSITIVITY IN PATIENTS WITH BILHARZIAL HEPATIC FIBROSIS

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ABSTRACT

Delayed hypersensitivity has been investigated in patients in different stages of bilharzial hepatic fibrosis. Skin tests, lymphocyte transformation tests and human leucocyte migration-inhibition tests were made in patients and in controls (healthy volunteers and persons with non-bilharzial hepatic cirrhosis).

A majority of bilharzial patients, grouped into three clinico-pathologic classes, showed significant reactions when evaluated by these three tests. There was no apparent impairment in delayed hypersensitivity in patients with varying degrees of bilharzial hepatic fibrosis. In addition, tartar emetic treatment had no influence on the results up to 2 weeks after completion of the therapy.

IMMUNOLOGICAL STUDIES IN BILHARZIASIS IN EGYPTIAN CHILDREN

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ABSTRACT

Phagocytosis and immunological responses are important mechanisms in combating foreign invaders. In parasitic infestations, especially when the invader passes into different stages of maturation and when the disease has variable clinical forms, it is expected that the response of the host is variable.

The study comprises 16 bilharzial subjects 4-12 years old, representing the two types of bilharziasis in their different stages, and 13 healthy controls.

Using the nitro blue tetrazolium (NBT) test both direct and stimulated and estimating serum immunoglobulins, the study revealed that: (1)

the absolute number and percentage of NBT positive neutrophils is low in controls; (2) the phagocytic power is significantly decreased in bilharzial patients especially in advanced cases with ascites; (3) in hematobiasis, urinary tract infection is not uncommon and a false positive or false high percentage of phagocytic power may be obtained, but when infection is treated the true markedly reduced phagocytic power of the polymorphonuclear leukocytes (PNL) is observed; (4) in mansoniasis, blood proteins, especially gammaglobulins, are affected markedly, especially when ascites is present. The conclusion reached is that estimation of phagocytic power of PNL and estimation of serum immunoglobulin can serve as indices of immunity in bilharziasis.

THE HOST ANTIGEN PHENOMENON IN MURINE SCHISTOSOMIASIS

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ABSTRACT

Using a recently developed microsurgical technique, accurately counted numbers of adult S. mansoni grown in hamsters have been transplanted to the mesenteric vascular systems of inbred mice immunized with hamster tissues. Despite serological evidence of anti-hamster antibodies there is no significant difference in the extent of worm destruction between immunized mice and non-immunized controls. Similar experiments involving the transfer of schistosomes between inbred strains of mice also show no evidence of increased killing by immunized animals. These results may indicate that the «host antigen» phenomenon demonstrated in rhesus monkeys may not operate in mice.

THE PROTECTIVE EFFECT OF ADULT SCHISTOSOMES ON SUBSEQUENT CERCARIAL CHALLENGE IN MICE

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ABSTRACT

The development of a microsurgical technique for the transfer of adult schistosomes to the mesenteric vessels of mice has allowed an accurate determination of the protective effect of known numbers of adult parasites to subsequent cercarial challenge. Preliminary results indicate that two pairs of transferred schistosomes provide a significant degree of protection on challenge with 50 cercariae in mice. Protection is only partial, however, and the egg-laying capacity of the two pairs of adult schistosomes results in the heavy infiltration of the livers with schistosome eggs, and death of a significant number of mice.

THE USE OF HOMOLOGOUS ANTIGEN IN THE INDIRECT FLUORESCENT ANTIBODY TECHNIQUE AND THE INTRADERMAL TEST IN HUMAN INFECTIONS WITH *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA HAEMATOBIUM*

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ABSTRACT

A group of persons infected with **Schistosoma mansoni** and a group infected with S. **haematobium** were examined immunologically with the Indirect Fluorescent Antibody Technique (IFAT) and the Intradermal Test (IDT). The patients *were tested simultaneously with S. mansoni and* S. **haematobium** antigen, in the IFAT by the use of juxtaposed sections of S. mansoni and S. haematobium worms, in the IDT by injecting in each of both arms one antigen. Increased affinity for the homologous antigen was expected to manifest itself in the IFAT by brighter fluorescence and in the IDT by a bigger wheal and/or induration. When a difference between the antigen sections of at least one titre was found with the IFAT, or when a difference in size between the wheals and/or induration of at least 0.05 cm^2 was found, the person was said to have preference for the antigen giving the highest titre, respectively, the biggest wheal and/or induration.

By testing with the IFAT the majority of the persons infected with S. mansoni or S. haematobium showed no preference, but those who did, did so mostly for the homologous strain. In the immediate response to the IDT the majority of patients infected with S. mansoni or S. haematobium showed preference, mostly for the homologous strain.

In the delayed response to the IDT, in both infections, preference was observed for the S. haematobium antigen, but, in the S. mansoni infections, to a considerably lesser degree.

In general, a tendency to preference for the homologous antigen was observed, being more clearly seen in S. haematobium infections than in S. mansoni infections.

THE PROTEASES OF *SCHISTOSOMA MANSONI* CERCARIAE AND THE CERCARIACIDAL EFFECT OF ZINC*

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ABSTRACT

Penetration of host skin by Schistosoma mansoni appears to be mediated by the secretion of proteolytic enzymes present in the pre-acetabular glands of the cercaria. Since an understanding of the nature and mechanism of action of these proteases might offer a new approach to chemotherapy of schistosomiasis, the proteases from cercarial homogenates and secretions were isolated and partly characterized by Sephadex Chromatography, sucrose gradient centrifugation and gel electrophoresis. The molecular weight of the major protease species is 25,000-28,000 daltons; it has an approximate sedimentation constant of 2.6S.

Studies with specific protease inhibitors were undertaken in an attempt to obtain agents which might be used therapeutically in preventing cercarial penetration. The cercarial proteases can be classified as serine-proteases since they are inhibited completely by DFP and PMSF; they are not inhibited by certain specific trypsin (TLCK) or elastase (Ac-Ala-Ala-Pro-AlaCH$_2$Cl) inhibitors.

However, inhibitors of both chymotrypsin and subtilisins, TPCK, Z-Phe-CH$_2$Cl and Z-Gly-Leu-Phe-CH$_2$Cl do inhibit cercarial protease activity (60% at 0.1 mM; 80% at 0.2 mM); further attempts to obtain specific inhibitors of the cercarial proteases were presented.

Protease activity was shown to be sensitive to the presence of divalent cations, being stimulated by low concentrations of Ca++ and Mg++ (0-10 mM) and inhibited at higher concentrations. Concentrations of Zn++ as low as 1 mM inhibit protease activity completely. In addition to its action on the proteases, zinc also affects cercarial morphology and longevity. The addition of ZnCl$_2$ to 0.5 mM decreased cercarial longevity from 10 hr (t 1/2) to 3.5 hr, while 5 mM ZnCl$_2$ decreased longevity to 1.8 hr. Extensive disruption of cercarial morphology was observed microscopically. We suggest that zinc may prove to be a simple, inexpensive and efficacious cercariacidal agent in the prevention of human schistosomiasis.

PAPERS PRESENTED BUT NOT READ

PRELIMINARY INVESTIGATIONS OF CELL-MEDIATED IMMUNE RESPONSES OF MAN IN SCHISTOSOMIASIS INFECTION

E.H. El-Raziky, Kouka S.E. Abdel-Wahab,
G. El-Attar and A.A. Ata

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CIRCULATING SOLUBLE ANTIGENS AND ANTIBODY IN SCHISTOSOMIASIS

M.A. Madwar and A. Voller

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IMMUNOLOGICAL STUDIES IN SCHISTOSOMIASIS WITH SPECIAL REFERENCE TO IMMUNE COMPLEXES

M.A. Madwar and A. Voller

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IMMUNOSUPPRESSION OF SCHISTOSOMAL GRANULOMA

M. Salah Ibrahim and H. El-Sherif

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STUDIES ON THE POSSIBILITY OF PRODUCTION
OF IMMUNITY AGAINST SCHISTOSOMIASIS
IN EXPERIMENTAL ANIMALS

S.M. Shoeb, K. Basmy, M. Madwar,

A. Masoud and M.F. El-Hawary

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HISTOPATHOLOGICAL STUDY ON THE IMMUNE RESPONSE
IN THE SPLEEN IN EXPERIMENTAL MURINE
SCHISTOSOMIASIS MANSONI

A.Y. Montasser and M.A. Abo-Hashish

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A STUDY OF SOME INTERPLAYING IMMUNOLOGICAL FACTORS
IN BILHARZIAL HEPATIC FIBROSIS

M.H. Ghanem, F.K. Guirgis, M. El-Sawy, S. Khalil,

F. Abul-Kheir and A.S. Mourad

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IMMUNOLOGICAL CHANGES IN DIFFERENT STAGES
OF HUMAN SCHISTOSOMIASIS

M.H. Ghanem, M.F.S. El-Hawary, I.A. Issa,

A.R. Wafy and A. Farag

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SERUM IMMUNOGLOBULINS IN MICE INFECTED
WITH *SCHISTOSOMA MANSONI*

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CELL-MEDIATED IMMUNE RESPONSE TO *SCHISTOSOMA MANSONI*
IN EXPERIMENTALLY INFECTED ANIMALS

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ECOLOGICAL AND HABITAT CONTROL OF SCHISTOSOMIASIS*

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Mr. Chairman, Ladies and Gentlemen, I am tremendously delighted to be here on behalf of the United Nations Environment Programme (UNEP) to participate in this very important Conference. We, all here present, are party to an event of great historical significance. Four thousand years ago, in this land, the parasitic disease of schistosomiasis was detected. Since then, it has defied the advance of science and technology and medicine. It is spread through three continents and infects millions of people. It is very heartening that our mutual concern over this unpleasant malady, and our desire to have it under control have brought us back to this land.

It is a pleasure for me, Mr. Chairman, to have this opportunity to thank formally on behalf of UNEP the countries which accepted the invitation particularly to join in the considerations on ecological and habitat control of schistosomiasis, the preparation of which WHO/UNEP have had the responsibility.

Specifically also, I should like to express thanks to the countries which do not have the disease, and to International Foundations which have so magnanimously accepted the responsibility of concern and come here to participate in our deliberations to find additional means of subjugating this disease.

It is my further pleasure and privilege to pay tribute to the UN organizations and particularly to WHO, to private institutions like the Rockefeller Foundations and the Edna McConnel Clark Foundation, to various national research institutions and to the many independent professionals and technical people who, for decades, with dedicated enthusiasm have studied and sought methods of ridding mankind of this disease. The information which has already accumulated from their extensive endeavours to control schistosomiasis provides a firm stepping stone on which to base plans for further approaches.

During the 2nd Governing Council of UNEP, distinguished delegates expressed their concern over the continued threat of animal and plant pests to human well-being. Taking account of on-going control programmes and mindful of their concern over the role of pesticides as environmental pollutants, the Governments specifically requested the Executive Director of UNEP to pursue the search for methods of control alternative to the use of chemicals which may be implemented in the interest of protecting human and environmental health.

Subsequently, last year, at a meeting held in Geneva which was attended by experts in the field of schistosomiasis, representatives of WHO, and the organising committee of this Conference, it was decided to hold, as part of this International Conference, a meeting, jointly organized by UNEP and WHO on the ecological and habitat control of schistosomiasis. In taking this decision, full cognisance was taken of the fact that this conference would consider in full, other aspects of schistosomiasis relevant to its control, thereby enabling bio-environmental considerations for schistosomiasis control to be viewed against chemotherapy, immunization and other forms of vector control within an integrated and multinational programme.

Mr. Chairman, in order to utilize to the maximum time to be devoted to the consideration of the subject listed under item 5 at this Conference, it was decided to depart slightly from the standard practice of having expert papers read at the Plenary Session.

During the preparatory phase therefore, we invited from Consultants, working papers on sub-items which had previously been identified as relevant to the subject of ecological and habitat control of schistosomiasis. We also requested from participating countries, country reports based on an outline, designed to provide comprehensive information which has relevance to the subject. At an Expert Meeting held in August in Nairobi with the Consultants, representatives of WHO, UNESCO and the Conference Committee, the working papers, together with an expert background paper prepared by UNESCO and country papers which had been received, were thoroughly studied.

From the material available, the Expert Meeting identified a number of principles which can form the basis for formulating a multinational plan of action. Following circulation to participating countries, and on the strength of comments received, the expert committee report has been amended. The report will form the Agenda and the basis of discussion in committee E which will consider ecological and habitat control of schistosomiasis.

It is intended that the consultations and deliberations of Committee E stress ecological considerations in a multinational control programme of schistosomiasis. From the proposals, we hope, will emerge a draft plan of action which will be presented to the final Plenary Session of the Conference.

This arrangement has been deliberately made to bring the consultants and country representatives together so that in a frank discussion the principles outlined may be examined against the background of the experience of the countries participating in the meeting.

Comprehensive and useful reports have been prepared by Brazil, Egypt, Kenya, Nigeria, Philippines, Puerto Rico through the U.S. Government, Sudan and Uganda. France and the Netherlands have also provided useful papers.

It is an honour for me, Mr. Chairman, to present shortly the report of the UNEP Expert Committee on the ecological and habitat control of schistosomiasis. The Report was prepared by Consultants of international repute and long experience in the field of schistosomiasis.

Now, Mr. Chairman, permit me the pleasure of introducing the Consultants.

Dr. Frederico Barbosa is Dean of the School of Health Sciences at the University of Brasilia, Brasil.

Dr. William Jobin is Head of the Biomedical Sciences Division of the Puerto Rico Nuclear Centre, Puerto Rico, U.S.A.

Dr. Peter Jordan is Director of the Research and Control Department of the Rockefeller Foundation in St. Lucia, West Indies.

Dr. Gladwin Unrau is a Sanitary Engineer by profession, but heavily involved in schistosomiasis control in the same organization.

Dr. Abdel Malek is Professor of Parasitology in Tulane University, New Orleans, U.S.A.

Dr. F. Eugene McJunkin is currently Vice President of the Environmental Services Corporation, Chapel Hill, N. Carolina, U.S.A.

Dr. Henry Van der Schalie is Professor of Zoology and Curator of Molluscs at the Museum of Zoology the University of Michigan, Ann Arbor, U.S.A.

I was particularly gratified to note the encouraging references made both at the Inaugural and Plenary Sessions to ecological and other bio-environmental methods of the control of schistosomiasis. I am happy that the director of the World Health Organisation, Dr. Mahler, shares our view of the absolute need to interweave health in the total development process using both the conventional and non-conventional weapons at our disposal. We are dealing with a tricky problem. We in UNEP believe however that a carefully planned strategy based on effective mobilisation of all available resources and an integrated action-oriented programme will get us somewhere.

The essential components of such an integrated action programme would, in our opinion, appear to include :

— destruction of the parasite in the human host, or interference with its egg producing mechanism and capacity, using chemotherapy ;

— prevention of contact between the eggs and the snail breeding sites. This would depend on satisfactory sanitation and waste disposal systems ;

— prevention of contact between man and infected waters. This would require safe water supply systems ;

— destruction of the snails and their habitats by various means which are environmentally safe so as to make them ecologically unsuitable for further proliferation of the parasite.

— protection, when we know how, through immunization.

Let me hasten to interpose that WHO, in the UN family system, has the overall responsibility of World Health. We in UNEP work on Environmental Programme. It is our business to be concerned about environmental impacts of existing methods of control of diseases. Mollusciciding is the best method we know for breaking the cycle of schistosomiasis and molluscicides are used sparingly in control — only a few parts per million. But, in spite of this, there is much evidence of the adverse effects of the chemicals on the habitat and its biota, which also form part of the intricately woven and delicately balanced human environment.

We therefore seek to encourage alternative methods which are drawn on ecologically sound approaches based on elements which lend themselves to serious consideration in the formulation of an integrated control programme. We consider that such a programme would also

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cover related factors, such as the presence of aquatic weeds which support snail populations, the limnological character of the medium, the mould and degree of exposure of the shoreline, the effect of wave and wind action on water movement, as well as a number of other relevant factors which may indirectly affect the success of the control operation.

We are further assured, drawing from past experience, that there should also be a concerted programme that would, in addition to using environmentally-sound methods to break the life cycle of the parasite, recognize the need for an equally «environmentally-sound» economic and social infrastructure to support the implementation of control programmes. Public awareness of the environmental implications of the disease and the existing methods of its control, popular participation in the implementation of strategies to combat the disease, and political will on the part of the international community to co-operate in eliminating or at least reducing the adverse effects of the disease are pre-requisites for the successful implementation of any plans to this effect.

With this in mind, the Consultants were invited to prepare papers which would assist in examining the ecological approach to control further. A paper was specifically requested on the side-effects of the use of molluscicides. The paper has indicated that information in this field is still meagre. Of the evaluations which have been made, it is known that molluscicides affects the biota to varying extents. But generally, destruction of the invertebrate microfauna which are essential links in the ecological food chain which feeds fish and other aquatic organisms does occur.

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There is evidence also to confirm the adverse effect of molluscicides on fish mainly because of the metal base used in most formulations. Bayluscide has been shown to be toxic to 18 species of freshwater fish. Frescon is also piscicidal at some concentrations and is some types of formulations. Yurimin kills fish and Tri-butyltinoxide (TBTO), is toxic to Tilapia and guppies which are believed to feed on cercariae.

Limited information on the effect of molluscicides on other fauna shows that amphibia and the large crustacean decapods (Potamon sp.) are not affected by copper sulphate ; but they succumb to other molluscicides. The estimation of the effect of molluscicides on mammals has been based, in most instances, on small numbers of laboratory animals and the observed experimental effects have been due to exposure to large doses. Hardly any information exists on chronic toxic effects caused by prolonged administration of the chemicals. It is necessary to obtain information and evaluate the effect on irrigated crops and plants, soil, microfauna and drinking water, the long-term effect of the technique of slow release and aerial application.

Also, because spread of schistosomiasis is aggravated by contact with water sources exposed to insanitary conditions, another working paper was requested to examine the part played by frequent contact with infected waters in the spread of the disease. The St. Lucia experience in this field was useful.

An account of projects which use environmental alterations to achieve control provides examples from various places. Attention was also given to the part that biological control and genetic manipulation can play in bio-environmental control schemes.

Predator/parasite relationships, snail competition and population interactions are among the subjects discussed in the working paper. The biological control programme of Puerto Rico provided a worthwhile input.

Because of the major effect of water management programmes and particularly irrigation schemes on the spread of the disease a special paper to consider modifications which may be made to irrigation schemes in order to minimise their effect on the spread of schistosomiasis was requested. The paper considers among others, storage reservoirs, canal design, agricultural practices, drainage and environmental sanitation.

In the paper on the use of mathematical models for planning control strategies, irrigation systems, flood control schemes and drainage systems which may be modified through engineering to minimise spread were considered. Given the right input, aspects of vector control may be effectively modelled.

A rather interesting paper on the use of ambient temperature for snail vector control has proposed a novel approach which I expect will be discussed with much interest in the Committee.

A limited number of the Consultant papers are available for distribution. The Consultants, I have no doubt will elaborate on the contents of their papers, as appropriate, in Committee E.

In the Nairobi Report for study by the Committee E of this Conference, the expert meeting has identified a number of principles on which formulation of pilot projects may be based. Mr. Chairman, let me explain. Although we refer to pilot projects, we in UNEP are not using the term in the conventional sense. We are interested in encouraging action

towards control. We are using existing and already tried principles to work demonstration projects in the field. We do not intend to launch into new projects to try the efficacy of unproven ideas for control. The list of items which the Consultants have identified is by no means exhaustive, including principles which have been tried and proved to be effective in control projects. They relate to the prevention of contact between man and infected water sources by the provision of safe, alternate and conveniently placed water supply, and the institution of physical barriers between man and the site of infection.

The report recommends also, the control of snail vector populations by methods which alter snail habitats or destroy them, and the direct destruction of snails as by the manipulation of water levels to strand them.

Few scientific achievements which have benefited mankind in a practical way have been unqualified miracles. Ecological bio-environmental control methods are far from being miraculous. One can envisage a number of difficulties and constraints. It is my hope that in considering a plan of action, we shall be mindful of the difficulties but not be daunted by them.

An important constraint which was readily evident at the meeting of the Expert Group was that knowledge on a number of aspects of other principles which might prove useful for control is hazy and incomplete. The meeting therefore identified a number of relevant subjects which may be put to further study to fill some of the gaps in the knowledge on which ecological control may be based. Again, it is not our intention to become a research funding organization. We have neither the funds or facilities for

it. However, it is our hope that Committee E will identify additional areas where research would clarify our thoughts. Above all we would hope that Committee E draws up a programme of priorities in applicability in relation to the pilot projects which may be formulated, identify those research areas where further work would improve control. We also would like the Committee to define training programmes and how they may be executed.

It is, further, essential to recommend a system of pooling of information now scattered in national and international institutions all over the world, in order to effect exchange of information. WHO does have a wealth of information on schistosomiasis control. We wish to concentrate on encouraging the gathering and dissemination of information relevant to ecological and habitat modification and other bio-environmental methods of control to evoke the interest of the scientists of the international community.

Mr. Chairman, Ladies and Gentlemen, we are faced with a problem which is as old as an ancient civilization, as widespread as the distribution of contaminated habitats of vector snails and infected people, as stubborn as the tenacity with which the male schistosome clasps and hangs on to its female partner and as seriously pathetic as the slow decay of infected people. We are looking for effective practicable solutions within the means of suffering people. It is not always easy to find such an answer. Certainly, success cannot be achieved without involvement of people.

We cannot look down on simple methods. In fact with the environmental approach, methods tend to be simple and unsophisticated. Maybe that even recommends them for integration in a multidisciplinary programme of control. The measures which have been outlined are simple. They do not claim by themselves to be the final answer. But they provide alternative and additional methods to existing ones which have become increasingly expensive to undertake.

Schistosomiasis is an old disease. It has almost become accepted in endemic areas as inevitable. It requires the toughening of will and dedication to a purpose to break its back. It requires concerted effort from many quarters.

If we can, at this Conference, come out with a workable plan of action for control, then indeed we would again have made history in this land where it all began 4,000 years ago.

Predator/parasite relationships, snail competition and population interactions are among the subjects discussed in the working paper. The biological control programme of Puerto Rico provided a worthwhile input.

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ECOLOGICAL AND HABITAT METHODS
IN SCHISTOSOMIASIS CONTROL

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Contents

Introduction

The transmission of schistosomiasis depends on three events taking place in the same limited aquatic environment : the access of schistosome eggs from human (in some cases other mammalian) excreta, the presence and persistence of suitable snail intermediate hosts and the immersion of human skin in the water once the extrinsic cycle has been completed. This environment is subject to variations due to many factors and in turn, it influences behaviour patterns of the human definitive host and the bionomics of the snail intermediate host. Any consideration of modifying the environment as a basis for schistosomiasis control must therefore embrace habitat modifi-

cation affecting human activities and changes in basic ecology which may prove inimical to the snail intermediate hosts.

The control of schistosomiasis may be achieved by breaking the cycle of transmission at any point. In practice, attention has over the last two decades been directed to the chemical destruction of snails and chemotherapeutic killing of adult worms in man. At the conscious level this has been first because such methods required only an efficient control organisation and limited assistance from the public at large and second because they were found to be in part successful. There may have been an unconscious analogy with the principal methods used in malaria eradication programmes.

The record of ecological and environmental approaches to schistosome control in the scientific literature was unexciting until recently ; however attention has returned to them for several reasons, including :

1. Their effect is persistent without continual re-application.

2. Recurrent costs are often lower than for other methods.

3. Health benefits may extend to other infective diseases.

4. Benefits outside the field of health may sometimes accrue, as in increased agricultural production.

5. Evidence of success in controlled epidemiological studies has accumulated.

6. Labour and funds are more interchangeable than in mollusciciding or chemotherapeutic programmes.

7. The approach lends itself to local or small-scale use.

8. Environmental concern has made people unenthusiastic about chemical control.

While the use of molluscicides is a clearcut phenomenon, ecological methods cover a range of habitat and consequential human behaviour alterations which form an untidy «package» and whose components are often not usefully evaluated separately in the field by simple trials ; they interlock very closely so that, for example, health education is meaningless unless environmental changes providing safe water have been made and, conversely, ecological changes may not reduce schistosomiasis unless combined with suitable human behaviour modification.

We here consider the types of schistosome control methods that can be considered ecological and their efficacy in control programmes, relating them to effects on other diseases and biological systems and to the community behaviour needed for their success.

Numerous methods of attempting to reduce schistosome transmission have been employed and the definition of what is 'ecological' is largely subjective. We shall exclude consideration of chemical means to reduce snail and schistosome populations — molluscicides and chemotherapy — except where their use interacts with habitat modification. We shall also consider environmental sanitation and health education only in outline, but will give more attention to their interaction with habitat approaches to control.

The areas for primary consideration in this section are therefore physical alterations to snail habitats, approaches using other organisms, and environmental sanitation.

2. The Basis of Ecological and Environmental Control — Methods and Techniques

We consider the techniques of environmental control separately from projects and pilot schemes in which they have been fully evaluated. Why is this ? There are many procedures which have been shown to decrease snail numbers in an area or which might be expected to reduce contact of people with potentially infective water. They are considered below. Far fewer environmental methods have actually been used in a human community and their effects on schistosomiasis in man determined, though this is the ultimate measure of control. The effects on man are more difficult to measure but are a more exacting test of ecological methods and are considered in section 3.

Environmental methods may be aimed at three points in the transmission cycle : reducing the number of intermediate hosts by habitat modification, reducing potentially infective human contacts with water, and preventing access of schistosome eggs to snail habitats. Biological control measures are briefly considered separately.

Environmental schistosomiasis reduction has two aspects : control of transmission that is already present and preventing the spread of transmission through water developments such as irrigation schemes and impoundments for hydroelectric purposes.

2.1 Reduction of Intermediate Hosts by Environmental Methods

Snail control is a rapid and effective means of reducing transmission of schistosomiasis and its efficacy is likely to be enhanced if combined with other methods of control. However, if used alone its effects on human schistosomiasis are manifest only slowly and also the extent of snail reduction needed for a useful lowering of transmission is uncertain. Studies of the ecology, bionomics and population dynamics of the molluscan host are axiomatic in the planning and evaluation of attacks on the snail habitat. The life-cycles of snail intermediate hosts, their infection and subsequent production of cercariae, and the transmission of infection are all affected by seasonal and climatic changes. Knowledge of these relationships must form the basis of the timing and application of any considered measures directed against snails in an attempt to control transmission. Failure to achieve a successful degree of control is usually attributable to a lack of basic information on such relationships and often to the dissipation of applied efforts through wrong emphasis or mis-timing. It is also important to consider that the factors responsible for transmission vary considerably and direct extrapolation of data on snail populations and their infections from one area to another, or data on the behaviour of the definitive host may be totally invalid, even in closely adjacent areas.

In many tropical areas fluctuations in snail population density and in the production of cercariae are as great as in temperate conditions, and transmission may be strictly limited and consequent upon the presence of water during only certain periods of the year. Appropriate snail control measures may only be necessary therefore at particular times and for limited periods. In some areas however, where the hydrology and temperature remain stable, snail populations and production of cercariae are maximal throughout the year, and any chemical measures directed against snails must be applied frequently, or more permanent measures must be instituted.

2.1.1 *Limitations of mollusciciding*

There are effective molluscicides available for the control of the molluscan intermediate hosts of human schistosomes. In many habitats however, the application of these compounds results in only a temporary reduction of the snail population and a temporary reduction in transmission of schistosomes. This is certainly the case in habitats within extensive watersheds and in water conservation and irrigation projects, where it is usually necessary to apply a molluscicide three or more times annually. Mollusciciding is most cost-effective where the volume of water to be treated per person at risk is small. It may therefore be well suited to arid areas where transmission is seasonal and confined to a few small pools. Conversely, it may be unsuitable in large rivers and lakes unless transmission is focal in distribution along parts, of the periphery. Where the population density is high, and the water volume per person is therefore low, mollusciciding may be appropriate, even though the total water volume is large. Irrigation schemes where water management is possible are also suitable for chemical control.

It has been estimated that the annual cost of molluscicide and its effective application in the 5.5 million acres under perennial irrigation in Egypt would be at least US $ 1.35 to 2.25 per irrigated acre. In Iraq, with 8 million acres under various types of irrigation and in an area with more pronounced seasonal differences and a shorter period of irrigation, it was estimated that molluscicide application would cost US $ 0.70 to 2.00 per irrigated acre. In the Philippines it was estimated that the molluscicide required for a single application in the known endemic areas, without the cost of application, would have been US $ 750,000 (McMullen, 1962). These costs have risen sharply of course

in the last few years. In Rhodesia, in streams and water conservation ponds, it was estimated that snail control with molluscicides costs approximately US $ 12.5 per 1000 acres, and in the Sudan the present annual cost of chemical control for the Gezira irrigation system (1 million acres) would be approximately US $ 1.6 millions. These large recurring expenditures cannot always be justified or sustained on economic grounds and careful consideration must therefore be given to developing different or complementary types of control including the reduction or control of snail habitats by environmental changes.

2.1.2 *Control of snail habitats*

In order to effectively change the ecology of a snail habitat so as to render it unacceptable, data on the ecological requirements of the snail must be available. If the specific ecological requirements are known, then relatively small changes may be sufficient ; but fundamental alterations are generally required in order to achieve the desired permanent result. The snail intermediate hosts tolerate relatively wide limits of different physical, chemical and biological factors. It is difficult to alter any one of these many factors sufficiently to effect adequate control, although alteration of one factor may subsequently influence others and achieve the desired effect. In relation to any aquatic habitat the removal of water is the most effective measure, and if this can be made permanent without adversely influencing local needs, it is certainly the most satisfactory available method. If, however, removal of water is only a temporary measure, as must be the case in irrigation systems, its efficacy will depend upon the ability of the snails in question to withstand desiccation. The pronounced capacity of aquatic planorbid

and bulinid snails to survive periods of drying must be considered in this case.

In natural breeding habitats, drainage is one of the most effective measures against aquatic snails, but often other alternatives such as increasing the velocity of water flow, stream straightening, deepening of marginal areas, elimination of pools, removal of vegetation and, in certain areas prevention of moderate organic pollution, will profoundly contribute to a reduction of snail breeding.

Control of the rate of flow and the means to fluctuate the level of water in storage dams, reservoirs, and irrigation systems, are important assets, and efficient water-management is an essential adjunct to any attempt to control transmission. In Puerto Rico the level of water was made to fluctuate in two small reservoirs and to fall fast enough to strand the snails and their ova. Fluctuations of 0.5 metres every 5-20 days during the snail breeding season can apparently control populations of *Biomphalaria glabrata*. Automatic syphon spillways can be used to cause these fluctuations at a reasonably low cost. The method is however only likely to be feasible in small or medium-sized reservoirs where the additional loss of water is acceptable (WHO, 1973).

The cost and justification of any permanent alteration of an environment to effect control must, of course, be established and the role of different habitats either as active transmission foci, or as reservoirs of snails for other natural foci, should be known. This may be very significant where fish culture or rice-growing is an important local practice and where the co-operation of the indigenous population must be obtained if effective measures are to be instituted.

The method of reducing habitats in control of snails was first put forward in Egypt by Leiper (1916) but rather discouraging results have been obtained in most attempts to use environmental techniques. It is considered that many of the attempts made to employ environmental techniques have failed because of the primitive nature of irrigation and agricultural practices often used in endemic areas (McMullen, 1962). Nevertheless, there are examples in the Philippines and in Japan where control of irrigation water, improved agricultural methods and adequate drainage have proved successful (Pesigan et al., 1958 ; Hairston & Santos, 1961 ; Okabe, 1957 ; Komiya, 1959 ; Okabe et al., 1967).

Many natural habitats in all endemic regions are important foci of transmission and their diversity calls for considerable ingenuity in devising different methods to effectively eliminate or reduce them. Adequate topographical, hydrological and geological data must be obtained and wet and dry season surveys made in order to locate all apparent foci and potential ones. The watershed must be carefully examined and attention given to large rivers, small perennial streams, seasonal streams and flash water-courses, seepages, lakes, marshes, swamps, ponds and temporary pools, and close attention must be paid to basic ecological factors including velocity of flow, flooding, aquatic vegetation, pollution and the existence of micro-habitats. Drainage and filling may not always be practicable and periodic pumping from channels or wells may prove satisfactory in drying out marshes and swamps. In some localities marshes have been eliminated by constructing ponds and using the excavated earth as fill (WHO, 1965) ; the method has been effectively used in the case of sluggish streams with swampy

edges in Leyte to control *Oncomelania quadrasi* (Hairston & Santos, 1961). This species cannot live in such ponds, and fertile soil is thus made available for cultivation. Small pools are important transmission foci of *Schistosoma haematobium* in some areas, being frequently used for domestic purposes and as watering points for animals. The most satisfactory form of control would be infilling, but this is generally impracticable, as is fencing, particularly if no alternative safe water supply is available for local needs.

2.1.3 *Avoiding creation of new habitats*

McMullen et al. (1962), noted that in arid and semi-arid areas the provision of water by irrigation, or the conversion of basin and partial irrigation to perennial irrigation usually results in an increase in the prevalence and intensity of schistosomiasis, because the same factors which make an endemic area more satisfactory for man also make it suitable for the molluscan intermediate hosts. In a few places however, like Musayeb in Iraq, no snails were found in the irrigated area and this was attributed to the water management practice. In this case measured amounts of water were delivered to individual farms for 5 days on a 24-hour basis, and then there was no water for 5 days. Other schemes in Iraq which had snail infestation were under no water regulation and seepage, over-irrigation and water-logging were common (McMullen & Rainey, 1959). A later survey at Musayeb in 1961 revealed that snails were present in certain habitats and limited transmission of *S. haematobium* was taking place. It is not known whether this was due to a change in the water-management routine or to biological adaptation in the canal system which permitted snail infestation in certain habitats.

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In Kenya the well designed and well managed Miwani Sugar Estate near Kisumu was apparently free of snail breeding and transmission of *Schistosoma mansoni,* which, it is considered, was attributable to : the well designed distribution and drainage systems on properly graded land ; water pumped from river to field laterals by pipe ; transportable siphons used to convey water to the field furrows ; soil moisture tests made before irrigation was permitted, giving an interval of about 15 days between relatively short irrigation periods ; labourers not dependent upon canal systems for domestic water ; modern equipment used for constructing and maintaining the system ; and herbicides used to assist maintaining the system free of weeds (McMullen, 1962).

In schemes in Ghana and Tanzania there is also some evidence that well-designed and constructed irrigation systems with efficient drainage, correctly prepared land, sound water-management, adequate maintenance and good agricultural practices have prevented the usual increase of schistosomiasis. The conditions found in many other schemes, however, including low gradient canal systems with much silt and vegetation, such as in the Gezira system, Sudan, with unsatisfactory water management schedules employed for conveyance systems, poor drainage channels with night storage dams and temporary pools, are such as to provide suitable conditions for widespread snail infestation. These factors, together with a lack of piped water supplies for domestic purposes, proper sanitation and the sitting of houses near irrigation canals, inevitably result in a considerably increased incidence of schistosomiasis (Webbe, 1963 ; Sturrock, 1965).

The application of engineering measures in water resource developments which are likely to minimise snail infestation and reduce transmission of schistosomiasis have been well reviewed (Ansari, 1973). Frequently however, expensive construction and materials must be justified on gorunds other than disease control. There is much evidence that the lining of canals is often not the complete answer to schistosomiasis control, but in general this tends to reduce snail breeding and minimise human contact, and make easier the control of residual snails by water management and/or chemical applications. In the Gezira irrigation system which has low-gradient canals, the annual cost of vegetation clearing is approximately US $ 140,000 and of silt removal US $ 700,000. It may be that consideration of increased capital investment in the construction of such schemes is indicated if possible recurrent savings of this magnitude could be made.

Man's activites either in relation to changes in flow of a water-course resulting in the creation of suitable habitats for the amphibious *Oncomelania* spp., or to the construction of dams for water storage, soil conservation or power production, resulting in the formation of suitable habitats for aquatic snails, should also be considered. In many areas schistosomiasis, like malaria, is a man-made disease. Numerous snail breeding foci are created by careless engineering practices associated with road and rail construction, bridges, causeways, and general industrial activity. The resulting ditches, borrow pits, quarries and pools frequently provide ideal snail habitats and eventually many of them become active transmission foci. The elimination of such habitats is the direct responsibility of the construction authority, and in most cases would involve little extra work or expense.

During the last 10 years public health problems associated with man-made lakes have become significant with the construction in Africa of the large impoundments such as Lake Kariba, Kainji Lake, Lake Nasser, Volta Lake and Kossou Dam, all of which now contain snail intermediate hosts of human schistosomes. The completion of the Cabora Bassa Dam in Mozambique and the Tafilalet Dam in Morocco will undoubtedly create new problems. Most of the impoundments, although primarily constructed for power production, are associated with different sized irrigation projects, and health problems associated with irrigation water as well with the impoundments, the spillways and the rivers below dams, must be considered. The developments taking place in the Mekong Valley in South-East Asia and those in the San Francisco Valley in North-Eastern Brazil will also entail *health* hazards from snail-borne and insect-transmitted infections, both in the impoundments and in associated irrigation systems.

On the shores of Lake Nasser in the desert and Lake Kariba situated in game plains, unsupervised immigrant populations exist in sufficient numbers to contaminate the lakes. On Lake Kainji, weed formation has not been a problem along the 350 km shoreline, but more than 300 fishing villages now exist and transmission of both *S. haematobium* and *S. mansoni* takes place (Brown & Deom, 1973). While transmission of *S. haematobium* is taking place in the body of Lake Nasser the main impact of this impoundment occurs as the result of perennial irrigation in upper and middle Egypt, and the water-logging of large areas there, con-

sequent upon increased irrigation and the high water table in some parts.

We know that there have been marked increases in the prevalence of schistosomiasis in certain man-made lakes. The changing ecology of these impoundments must therefore be closely observed, and human settlements and activities considered if improvements in human ecology and water management are to be made as a basis for feasible long-term control of the problem of schistosomiasis and other water-borne infections.

2.2 Reducing Human Contact with Infective Water

A second group of environmental alterations aim to keep people out of water that could be infective. This involves providing alternative water that is more convenient and attractive to the user as well as safe. It has been shown (White et al., 1972) that rural water users in the tropics show marked discrimination in their choice of water sources, though their quality standards are based on taste and appearance and disregard aspects of pollution through unfamiliarity with the germ theory of disease. So education must accompany improved water.

To affect schistosomiasis, more than a basic domestic tap or nearby standpipe is needed. It must be possible to wash clothes, and also in the hot tropics children will still swim in the natural water unless an alternative is provided. It may be necessary also to fence off the infective stream or pool. Such comprehensive improvements are expensive and only applicable to nucleated settlements. They have been used in South Africa, and also in St. Lucia where there is clear evidence of reduced *S. mansoni* transmission by this means. There is evidence that such measures do specifically reduce domestic and recreational contacts with infected

water, though occupational contacts will be unaffected. The capital costs of installing piped water in houses will vary with the situation involved, the distance from a water source and the settlement density. Supplies of this kind recently installed in St. Lucia cost $ 7 to $ 11 per head, including a single tap per household, a community laundry unit and a swimming pool. Such a supply will involve a high capital cost and maintenance expenditure, but it represents an important facility that will confer a general improvement in health with long-term lasing effects. A village standpipe water supply is unlikely to effectively reduce contact with infected waters but may provide some benefit. The improvements in domestic water supplies are unlikely to interrupt transmission except in particular epidemiological situations, but such installations may be very acceptable to health administrations as part of a broad rationale of public health control rather than a programme directed only against schistosomiasis (WHO, 1973).

Where a new settlement is being constructed, as for an irrigation scheme, dwellings should be sited away from canals and drains, and a safe domestic supply provided and maintained. This is obvious, but is often not done.

2.3 Reducing Access of Schistosome Eggs to Water

Evironmental methods to achieve this are basic sanitation which is used. The approach has had a poor reputation deriving from the work of Weir et al. (1952) in the field and Macdonald (1965) using mathematical models. It has been argued that the former did not achieve latrine use and the latter depends on some shaky assumptions and we concur with this view. Adequate assessment is awaited, though it is generally accepted that

S. haematobium is unlikely to be controlled in this way. Water-borne sewerage is too expensive and bore-hole latrines readily become unpleasant to use. Only in cultures of S.E. Asia where human faeces are a valuable resource, is sanitation a really hopeful approach. In China, where nightsoil conservation is long-established practice, improved leak-proof storage till ammoniacal destruction of schistosome eggs has occurred seems to have become widespread.

In the Philippines the use of a combination of improved agricultural methods, drainage, and the control of irrigation water has given promising results. In the area under control the snail population reduction has averaged more than 95% ; many habitats have been eliminated and those remaining made more amenable to chemical control (Hairston & Santos, 1961 ; Pesigan & Hairston, 1961). Land that had not been productive is now very valuable and is contributing to the local and national economy. The benefit of a change in farming practice has been demonstrated in that where rice was formerly grown by ponding, the number of snails was reduced from 200 to less than one per square metre and the crop yield increased by more than 50% after the adoption of intermittent irrigation (WHO, 1973).

McMullen (1961) noted that certain advantages and disadvantages accrue from the use of the measures already discussed. Local labour and materials can frequently be used. Waste land may become utilized and productivity thus increased. Improvements in irrigation and agricultural methods usually result in decreased snail population densities and an increase in crop production. The areas where it is necessary to apply molluscicides will be considerably reduced. There are however also serious limitations to such measures, since a high degree of co-operation must be generated between the executive agencies and the local population.

2.4 Biological Control

One ecological approach to schistosome control considers the use of other organisms to attack the snail phase of transmission. This has been done by predation, competition, or parasitism. A detailed account has been given by others. Schistosome larvae may also be attacked free in the water or by competition within the snail host.

Among the predators of schistosome-host snails are ducks, some fish, some other snails (considered below under competition), sciomyzid flies, and a range of other vertebrates and invertebrates. Most are relatively non-specific feeders so that although considerable reduction of high snail densities may result, the efficacy of predation falls off at low densities. The *sciomyzid flies are more restricted in food range* to snails but are not species-specific. In the sub-tropics where much schistosomiasis is present, marked seasonal cycles in both snails and predators may further limit efficacy of predation.

Competition between snail species for habitat may depend on food as a limiting resource, but grades into predation when egg masses of another species are deposited on the plants eaten. Again it is a relatively non-specific matter. This is also true of the various bacteria and other micro-organisms pathogenic to snails. However, recent evidence on *Helisoma* and its ability to displace bulinid snails is more hopeful.

Competition for habitat *within* the snail host has been shown for several trematode larvae.

Several features are common to all the work on biological control. In laboratory experiments using very simple habitats structurally and biologically, there is usually great reduction in schistosome larvae or snail hosts .Trials in outdoor small habitats have been variable, with considerable success in some habitats and less in others. This may lead to a more restricted view of the circumstances under which the control agent might be used than was originally entertained. The methods have not been used in epidemiological trials in nature using changes in human infection to assess their efficacy. One possible exception is *Marisa* in Puerto Rico, but a series of other interelated variables exist, so that the biological control effects cannot be disentangled from the other possible causes of the decline in *S. mansoni* which has occurred there.

Except in the small number of cases where complete displacement of one host snail by another mollusc has been described, it is hard to extrapolate from small field trials of effects on snail populations to the likely effects on infection in man, since very little is known of the relation between changing snail densities in the field and transmission. In general there is a low specificity to the biological agents described so that they fall off in efficiency as larvae or snail numbers decline ; analogies with biological control of insect pests is rarely close. However, this rather reserved view of the present position should be taken as an indication for more, though relatively long-range, research rather than for despair.

3. Control Programmes and Projects

There are few pilot projects in which ecological methods have been used and evaluated in a controlled manner against other approaches and against comparison communities, using schistosomiasis in

man as the measure of success. The nearest to successful evaluations are the recent work in St. Lucia, well described in the papers for this meeting by Unrau & Jordan (1978), and the earlier Leyte project on *Schistosoma japonicum* (Hairston & Santos, 1961).

Other programmes that appear to have been notably successful but for which rigorously controlled data are not available have been carried out in Japan, Venezuela, South Africa and Rhodesia, also in Iran and China (WHO, 1973).

4. Redefinition of the Problem

The preceding sections have shown that there are many ecological methods which can be used to influence schistosome transmission and that these have, in some circumstances, been effective in practical control pilot programmes. There are also many occasions when success has not been formally demonstrated and where no lasting benefits seem to have accrued. To answer the question 'is this method effective' is no longer enough as a guide to practical public health. We need to ask two further questions : 'under what circumstances can we expect a particular method to reduce schistosome transmission' and 'what complementary inputs are needed if this method is to prove effective.' To analyse this let us consider three contrasting real situations in outline.

4.1 A Successful Campaign against *Schistosoma japonicum*

A prolonged campaign in the five areas of Japan where *S. japonicum* was endemic before 1950 has been highly successful in reducing the amount of disease and the extent of *Oncomelania nosophora* habitat. Irrigation ditches have been lined with concrete and maintained clear of silt, vegetation and detritus. Molluscicide is

used in addition. The use of faeces as manure has been displaced by chemical fertilizers which may also affect snail populations. Land reclamation by drainage and filling has proceeded rapidly and the rice fields have been displaced by fruit. As a result, the prevalence of *Schistosoma japonicum* has been drastically reduced during the past 25 years. In 1956 the intermediate host, *Oncomelania nosophora,* was reported to breed in an area of 18,000 hectares, and the population at risk was 370,000 ; by 1966 the breeding areas had been reduced to 7,333 hectares and the population at risk reduced to 42,751 (Yokogawa, 1970). Molluscicides have been extensively used in conjunction with intensive habitat reduction and land reclamation measures. Irrigation ditches in rice fields have been lined with concrete and more than 80% of these channels have now been lined in almost all the endemic areas, totalling nearly 2000 km. The cost of concrete lining was between US $ 10,000-20,000 per km, and between 1966 and 1970 an average of 120 km were lined per year. These costs were borne by the local authorities and the national government. Reclamation of swampy areas by drainage and filling has been carried out (Okabe et al., 1967) and the reclamation of rice fields for the construction of housing areas has reduced the habitats of *Oncomelania* from year to year in most endemic areas. During the past 10 years there has been a gradual reduction in the use of native cows in rice fields in the endemic areas which were considered to be the most important reservoir hosts of *Schistosoma japonicum,* and wild rats probably now contribute to the maintenance of the disease. The national control programme has undoubtedly been very successful in Japan but socio-economic factors such as rising living standards and the effects of urbanization and industrial activity must also be considered (Yokogawa, 1972).

Improved water supplies and excreta storage facilities are now widespread and many smallish settlements have swimming pools. Reservoir host cattle have been replaced by tractors. General education is widespread to a high level and includes the natural history of diseases and their prevention whilst mass health education is facilitated by almost every household now owning a television set.

This is a highly effective programme at extremely high per capita cost. Several components were not primarily antischistosome measures and one may speculate that the disease would have declined in due course without a specific campaign, though this does not in any way detract from the excellent work of the public health authorities.

4.2 An Apparently Successful Campaign against *S. japonicum*

In mainland China *S. japonicum* has also been greatly reduced in prevalence according to such data as are available. The methods have included destruction of snail habitat by labour-intensive methods at low capital cost. The main techniques have been to bury snails by relining canals or filling them in completely after placing the infected surface layers at the bottom of the channel. New canals are then dug parallel to the old. An improved system of faecal processing has been widely adopted, whereby raw material does not come into contact with irrigation water, and faecal storage until the ammonia from urine has killed the schistosome eggs takes place in sealed earthenware containers not subject to flooding. Even fishermen are said to conserve faeces and avoid pollution of watercourses. The rigid community discipline here appears to have produced an effective

programme at low per capita cost, though detailed epidemiological evidence is scanty.

4.3 Problems of Water Development and *S. haematobium*

In recent years a large man-made lake resulted from a hydro-electric scheme involving a dam across a major river in Africa, the Volta. Thought was given to the risk of schistosomiasis prior to construction and it was hoped to restrict this by minimising the lake-shore population and providing adequate water supplies to settlements in the area. In the event, the lake was rapidly colonised by *Bulinus t. rohlfsi,* a snail species different from any which had been expected ; very large populations of immigrant fishermen from other areas endemic for *S. haematobium* set up settlements around the lakeshore, while maintenance of water supplies for the villages of displaced inhabitants was inadequate. Intense transmission around the lake has resulted and environmental approaches to control have not been successful as yet. Mollusciciding of focal areas near villages and chemotherapy are being undertaken.

These three sketches illustrate the complexity of achieving and planning ecological approaches to schistosomiasis control. Three groups of considerations are particularly important and it is to these that we wish to draw attention. The first concern *the types and distribution of costs,* obviously crucial and deserving analysis. Second are the *linkages* between schistosomiasis control and other activities and goals of the community. Third is the type and intensity of *community structure and discipline.* These three, and particularly the latter two, seem to us to determine the choice and likely success of ecological and habitat methods of

schistosome control. They have been inadequately considered in this context.

5. Determinants of the Choice and Efficacy of Ecological Methods

5.1 Costs

The per capita cost of the Japanese control programme is between 10 and 100 times greater than the Chinese one, yet both have had considerable success. This is on actual cash expenditures. But there are other differences. The bulk of Japanese spending is on concrete lining for irrigation canals, with a high capital outlay for construction costs. Recurrent costs for molluscicide and canal clearing are much lower. The cost of the Chinese environmental work comprises labour almost entirely, and this is contributed directly by the agricultural community. Since it is often not paid labour and is contributed under conditions where the marginal opportunity cost of labour is very low, great economies result. There are thus both trade-offs between capital and recurrent costs of environmental changes and also between expenditure on materials and availability of labour. The time-scale of expenditure is an important variable therefore. The time-scale of benefits is also crucial. Environmental methods can reduce transmission ; the adult worm population is unaffected directly and since the worms are long-lived it may be several years before benefits are apparent to the inhabitants of the area where they are applied. Chemotherapy has an immediate effect. Also, most environmental methods are more demanding of cooperation from the people than is mollusciciding, as will be discussed fully under 5.3 below, so that most environmental approaches have a long time-scale in mind rather than dramatic results in the short run.

5.2 Linkages

A molluscicide simply kills snails : any other result is a side-effect and probably an undesirable one at that. Installing a water supply would be expected to reduce many other diseases and to reduce the work of the women who were carrying water. It has multiple other good effects, or positive linkages, as well as reducing schistosomiasis. Ecological and habitat methods of schistosome control often have many linkages, both positive and negative. The latter need careful thought, the former add to the value of ecological methods and also costs of the procedures can be offset against these other advantages. Several linkages are considered here :

5.2.1 *Environmental control of multiple vectors*

Where several diseases spread by water-related invertebrate hosts occur in the same area, their ecology needs consideration to ensure that measures aimed at reducing one host do not favour increase of another.

A major feature of malaria control in the southern U.S.A. has been variation of reservoir levels to decrease mosquito breeding. Jobin (1970) and Jobin & Michelson (1969) have shown that similar fluctuation of water levels can be used to control *Biomphalaria glabrata,* host of *S. mansoni* in the New World. However, transferring this approach to sub-Saharan Africa, whether or not it had an appreciable effect on snail host of schistosomes, might well increase habitats suitable for *Anopheles gambiae,* the main vector of malaria there.

In raising stream velocities and installing dam spillways in measures to discourage snail breeding it is necessary to avoid creating habitats for the larvae of *Simulium,* vector of onchocerciasis.

More hopefully, the various measures which reduce snail breeding in canals will tend usually to reduce mosquito breeding and have a beneficial effect on the diseases they transmit. Borrow pits are more important as anopheline breeding sites than as snail habitats.

5.2.2 *Ecological control and conservation*

Over the last decade a picture of the environment has grown up with chemical pesticides considered as destructive, undesirable and to be avoided as far as possible, contrasted with ecological and environmental methods as gentle and more 'natural.' Such a simple view is misleading when applied to schistosomiasis. The distribution and abundance of snails tends to be reduced by those environmental changes which sharpen the demarcation between land and water : draining marshes, lining canals with concrete and keeping them clear of vegetation, using overhead irrigation to reduce the need for drains, filling in ponds and borrow pits. This makes the environment less rich and less diverse biologically by reducing the variety of habitats. Consequently wildlife is also less varied. Thus, while molluscicides have a dramatic lethal effect, the snail and other invertebrate populations soon return to previous levels if the molluscicide is not re-applied. Environmental changes produce long-term effects on the biota, often more profound than molluscicides but less apparent because sustained.

To say this is not to argue for one or other approach to schistosome control but rather to emphasise that simple polarization of the issues is both misleading and no guide to action in itself. It also points to an issue that has rarely been discussed : the potential conflict between environmental measures of disease control and conservation of nature. If the

intention to drain the Pontine marshes (for malaria control) had first been raised in the present decade, there would have been strong conservationist objections. The 'romantic' landscape is far more suited to schistosome transmission than is the renaissance or classical ordered landscape ! Hygiene and romanticism readily come into conflict. This will need open discussion before long and perhaps needs some thought even now.

5.2.3 *Agriculture*

Major alterations of snail habitats, particularly of *Oncomelania,* have large effects on the availability and productivity of agricultural land. A useless swamp may become a good rice-field or control of sluices may conserve water, reduce snail populations and increase crop yields. Such multiple effects make a schistosome control programme more acceptable to farmers. The benefits can be offset against the cost of the measures taken.

5.2.4 *Control of other diseases related to water supply and sanitation*

Environmental approaches centred on water supplies may affect very many water-related diseases (White et al., 1972). The improvement of water quality will reduce the strictly water-borne infections such as typhoid, cholera and some forms of diarrhoeal diseases. Making water more available, to discourage people from going to schistosome infested natural water, will reduce the incidence of diseases of low personal hygiene including skin sepsis, scabies, trachoma, among the superficial infections and the diarrhoeas and dysenteries. Such other infections, besides schistosomiasis, which depend on an aquatic intermediate host, for example guinea-worm, may be greatly reduced while the densities and access to people of vector insects which breed

in water (see above) would be decreased to some degree. Water supplies by themselves are, in most situations, relatively less effective against schistosomiasis than against several other infections, but when combined with health education and effective excreta disposal schistosomiasis may be substantially reduced. Latrines that are safe and are utilised (and this is a large step) will not only reduce *S. mansoni* and *S. japonicum,* with much less effect on *S. haematobium,* but may reduce *Ascaris* and *Shigella* prevalences also, together with other faecal-oral infections.

5.3 Community and Environment

Differing techniques of schistosome control make varying demands on the community. Massive concrete lined canals are provided 'for' the community who at most acquiesce — even if they object it makes but little difference — mollusciciding requires a well organised and disciplined small team of men who need to continue efficient action over many years, together with acceptance by the whole community. The Chinese approach necessitates strong active co-operation by everyone in the community. We believe it is not without significance that some of the most successful results from ecological and habitat methods at reasonable cost have come from three countries where a very strong discipline is either imposed on the communities or else strong internal community discipline and presence on the individual to conform are apparent : South Africa, Rhodesia and China. Often habitat and environmental methods are far more demanding of the community than is mollusciciding or even chemotherapy. We would suggest 3 types of relation between environmental control and community :

a) High capital cost imposed solution, low in community demands. However

almost everything needs *some* maintenance and all too often it is not provided. A tube-well is a simple water source, but the pump occasionally needs repair ; even a concrete-lined ditch needs some cleaning out.

b) Techniques requiring regular effort over many years by a small trained unit with minimal co-operation from other people. Mollusciciding is a good example and snail control by irrigation water management is another.

c) Techniques requiring less skill but wide community participation.

Type (b) is effectively an 'imposed' solution, asks less of human nature, but is precarious. Type (c) requires a highly disciplined community.

6. A Framework for Decisions

We have not suggested numerous new ecological methods for schistosomiasis control needing research, since that does not appear to us the first priority. We have indicated the importance both of community behaviour or discipline and of linked effects of habitat methods on other diseases and events. The implications for planning schistosomiasis control are clear. A wide view must be taken to fit the circumstances. It would be futile to attempt a very labour-intensive self-help type of habitat modification in a country with a small but competent vector control service not comprised of local people, and conversely it would be equally unsuccessful to attempt concrete lining of irrigation canals in a very poor country with a labour surplus. The time course of expected benefits should be made clear and, unlike the early stages of disease eradication schemes, care should be taken not to 'oversell' any habitat modifications. Improvements in sanitation and water supply should arise

from within the community as far as possible while snail habitat *modifications* should be made part of the agricultural pattern.

In general, ecological methods are the long-term hope for schistosomiasis control and should be applied steadily, and often in conjunction with other techniques of more rapid effect. Concomitant inputs of education are important and maintenance of any environmental works is crucial.

7. A Note on Research Needs

What are the main research needs ? There are several :

1. Increasing our understanding of the ecological epidemiology of schistosome transmission to the point where prediction is useful. This is rarely the case as yet : who thought that *B. truncatus rohlfsi* would invade Volta Lake ? Our understanding of which introduced snails might displace or reduce schistosome hosts is very small. This work requires (a) continual increase of background knowledge but also (b) strong support of any new and penetrating ideas on molluscan ecology, since precise understanding is lacking.

2. More empirical studies of the effect of environmental and ecological control measures not only for their own sake but also because the absence of such studies tends to discourage people from seriously considering them.

3. More adequate analysis of the social circumstances under which environmental methods may be implemented and of the cultural inputs required to achieve success.

4. Development (scarcely research) of low-cost methods for achieving en-

vironmental changes for irrigation, water supply, and sanitation, especially where local materials can be used. 'Self-help' approaches need development where the social milieu is suitable.

More important at present than research needs are training of staff and development of practical programmes in areas where environmental measures would benefit health by control of both schistosomiasis and other diseases.

LITERATURE CITED

Ansari, N., (editor) 1973. «Epidemiology and Control of Schistosomiasis» pp. 591-593. S. Karger, Basel — München — Paris — London — New York — Sydney.

Brown, A.W.A. & Deom, J.O., 1973. Health Aspects of Man-made Lakes. Symposium on man-made lakes, Knoxville, Tennessee, USA, 3-7 May, 1971. Geophysical Monograph Series 17: 755-764.

Hairston, N.G. & Santos, B.C., 1961. Ecological control of the snail host of Schistosoma japonicum in the Philippines. Bull. Wld Hlth Org. 25: 603-610.

Jobin, W.R., 1970. Control of Biomphalaria glabrata in a small reservoir by fluctuation of the water level. Amer. J. trop. Med. Hyg. 19: 1049-1054.

Jobin, W.R. & Michelson, E.H., 1969. Operation of irrigation reservoirs for the control of snails. Amer. J. trop. Med. Hyg. 18: 297-304.

Komiya, Y., 1959. The natural habitat of Oncomelania nosophora, the vector snail of Schistosoma japonicum and the present situation of the cemented ditches. Jap. J. Parasit. 8: 923-931.

Leiper, R.J., 1916. Observations on the mode of spread and prevention of vesical and intestinal bilharziasis in Egypt. Proc. roy. Soc. Med. 9: 145-172.

Macdonald, G. 1965. The dynamics of helminth infections with special reference to schistosomes. Trans. roy. Soc. trop. Med. Hyg. 59: 489-506.

McMullen, D.B., 1961. Intramural aspects of schistosomiasis control. Proc. Okla. Acad. Sci. 41: 1-5.

McMullen, D.B., 1962. The modification of habitats in the control of bilharziasis, with special reference to water resources development. In: Ciba Foundation Symposium on Bilharziasis — London, Churchill: 382-396.

McMullen, D.B., Buzo, Z.J., Rainey, M.B. & Francotte, J., 1962. Bilharziasis control in relation to water resources development in Africa and the Middle East. Bull. Wld Hlth Org. 27: 25-40.

Okabe, K., 1957. Schistosomiasis in Japan. J. Parasit. 43: Suppl. p. 30.

Okabe, K., Kifune, T., Shiraishi, S. & Takao, Y., 1967. Disappearance of Oncomelania hupensis nosophora populations in the Chikugo river bed after the artificial alteration of the vegetation. 1. Disappearance of the snail population in the neighbourhood of Mamazu, Saga Prefecture, J. Kurume Med. Assoc., 30: 1150.

Pesigan, T.P. & Hairston, N.G., 1961. The effect of snail control on the prevalence of Schistosoma japonicum in the Philippines. Bull. Wld Hlth Org. 25: 479-482.

Pesigan, T.P., Farooq, M., Hairston, N.G., Jauregui, J.J., Garcia, E.G., Santos, A.T. & Besa, A.A., 1958. Studies on Schistosoma japonicum infection in the Philippines. 3. Preliminary control experiments. Bull. Wld Hlth Org. 19: 223-261.

Sturrock, R.F., 1965. The development of irrigation and its influence on the transmission of bilharziasis in Tanganyika. Bull. Wld Hlth Org. 32: 225-236.

Unrau, G.O. & Jordan, P., 1978. Results of a water supply scheme on the transmission of Schistosoma mansoni. Proc. internat. Conf. Schist., Cairo.

Webbe, G., 1963. Known transmission patterns of Schistosoma haematobium in Tanganyika and the possible influence of irrigation on incidence of infection. E. Afr. med. J. 40: 235-9.

Weir, J.M., Wasif, I.M., Hassan, F.R., Attia, S.D.M. & Kader, M.A., 1952. An evaluation of health and sanitation in Egyptian villages. J. Egypt Public Health Assoc. 27: 55-122.

White, G.E., Bradley, D.J. & White, A.U., 1972. «Drawers of Water». University of Chicago Press: 151-222.

World Health Organisation, 1965. Snail control in the prevention of bilharziasis. Monograph Series No. 50, WHO, Geneva.

World Health Organisation, 1973. Schistosomiasis control. Technical Report Series No. 515, WHO, Geneva.

Yokogawa, M., 1972. Control of schistosomiasis in Japan. In: «Proceedings of a Symposium on the Future of Schistosomiasis Control», Tulane University, New Orleans: 129-132.

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THE USE OF MATHEMATICAL MODELS AND SYSTEMS ANALYSIS
AS GUIDES FOR SCHISTOSOMIASIS CONTROL MEASURES*

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* This working paper was prepared for the meeting on Ecological and Habitat Control of this
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(such as References cited, etc.) it is published as it stands.

Introduction

Two distinct approaches have been followed in the development of mathematical models related to schistosomiasis, one being the simple model of a limited phase of schistosome transmission or control, and the other being the rigorous development of comprehensive models of the entire transmission cycle. Perhaps Hairston's (1965) model of age-specific prevalence curve is the best example of the first ; Nasell's recent theoretical approach represents the latter. The theoretical approach is of long range interest but the more limited models have immediate utility in interpreting epidemiological data and planning control programs. While both approaches are important, here the limited models will be discussed since even their validation requires an immense amount of field work.

Of interest to public health authorities is the method developed by Paulini for «Allocating Funds for Molluscicides and Drugs in Schistosomiasis Control» where he evaluates the rapidity and economics of varying combinations of control methods. This is useful for careful planning in a new program to determine the wisest use of scarce funds. This paper shows the potential value of these models in planning control programs and selecting detailed strategies. While crude, the mathematical formulations make it possible to consider a large variety of strategies. The accuracy of predictions by the models however depend on their careful construction and verification against field observations.

Use of Models for Planning Control Strategies

Previous studies on population dynamics of planorbid snails made it possible to develop a fairy precise mathematical model of snail populations in lakes and reservoirs. This model has been verified with data from Africa and Puerto Rico and thus it can be used to evaluate snail control strategies.

Timing of Snail Control Effort

When organizing a program based on control of snails, a question to be answered early in the program is whether timing of the control measures is important. In the case of molluscicides, is there a month or season when the cost/effectiveness ratio is unusually low ? If not, timing is unimportant, but if the cost/effectiveness can be minimized by applying chemicals at a specific time of year, this should be designed into the program.

Selection of the best timing cannot easily be done experimentally since it involves large numbers of field tests with expensive and time-consuming evaluation. A simpler method is to obtain information on population dynamics of the snail and on seasonal characteristics of habitats typical to the area, and then to rationally evaluate the effect of control measures at different times of the year. To assist in this mental exercise, a computer program for modeling a snail program has been developed (John & Michelson, 1968). Its reliability has been previously tested (Fig. 1). The data were for a population of African snails, since comprehensive field studies were not available at the time the model was developed.

The manipulation of the model for the purpose of predicting population histories is accompanied by proposing increased death rates for simulation of application of a molluscicide, desiccation, and other catastrophic events. For a given species the number of survivors in a group, over a given time-interval is

$$s = s_0 \times p_a \times p_c$$
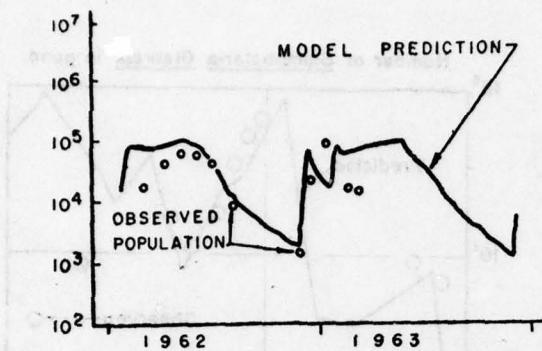
Fig. 1. Verification of snail population model against field data from study on a *Bulinus globosus* habitat in Africa (Foyle pond).

where s_0 is the original number of snails, p_a is the age-specific survival rate for the time interval, and p_c is probability of surviving any catastrophic event which might have occurred in the time interval.

Model of Snail Host Population

Description of the mathematical model. The computational model of a population of aquatic snails was written in Fortran IV. The model required input information on the snail species (age-specific survival rates, age-specific birth rates, a fecundity factor, the relationship of fecundity to temperature, the volume of the crowding zone), and information on the habitat (volume, temperature, and food). From these data a 3-year prediction of the snail population was calculated. The population was described by the model for 10-day intervals in terms of the total number of snails, and number of eggs.

An iterative process was used for the computations. The computational program began with a specified population, grouped into 10-day age intervals. The first step was to calculate the number of snails surviving to the next time period for each age-group. The time-period and the age-interval were set at 10 days to approximate the hatching time

for eggs of *Biomphalaria glabrata* and *Bulinus globosus*. The number of snails was then calculated for the first period, both for output and for use in crowding calculations. The next step involved calculation of various parameters for the first time period, such as average age of the population and food density in the habitat. After testing for population crowding, the egg production was calculated for each age-group, according to the age-specific fecundity factor and water temperature. If the population was not crowded, fecundity was proportional to food density, whereas if the population was crowded, fecundity was calculated as being proportional to the ratio of food density and the number of snails per crowding zone (Jobin & Michelson, 1968). The number of eggs produced was then summed for the first time period, reduced according to hatching and catastrophic death rates, and transferred to the first age-group of the next time period. The entire process was then repeated, advancing one period at a time.

Since the original development of the model several field studies have been completed on *Biomphalaria glabrata* (Jobin, 1970). To determine if the model was also reliable for *B. glabrata* and thus useful for Brazilian conditions, the field rates of survival and the environmental conditions from Pond B in the Puerto Rican study were used in the model to predict the history of the snail population (Fig. 2). The prediction agreed quite well with the observed number of snails. In a similar manner the environmental data from Pond C of the same study was put into the model (Fig. 3). Again the predicted number of snails was quite similar to that actually observed in pond. It was therefore assumed that the model is reliable for predicting population histories of the snails in Brazil, or elsewhere, with the proper data (Fig. 4).

MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A

Number of *Biomphalaria Glabrata* in pond
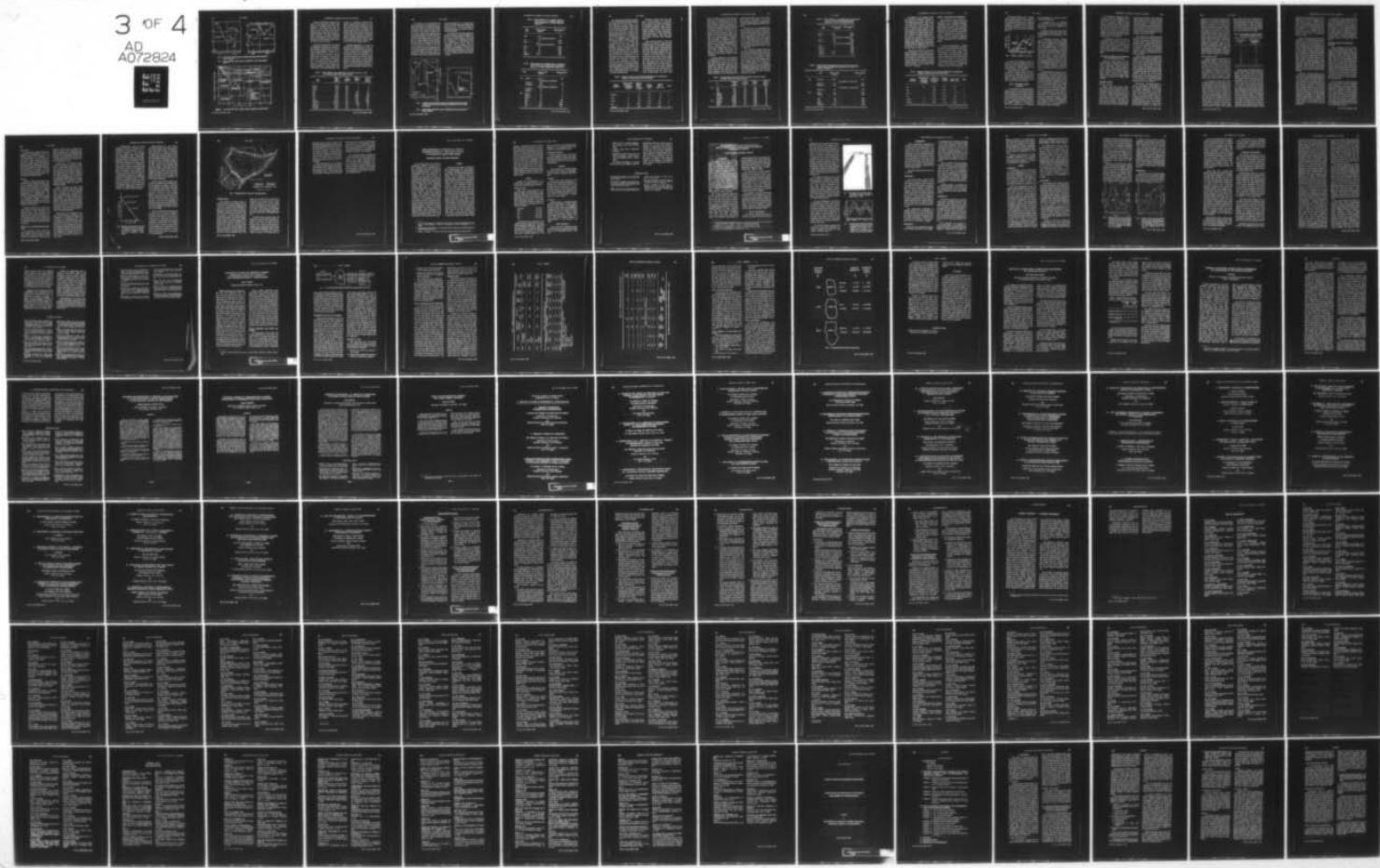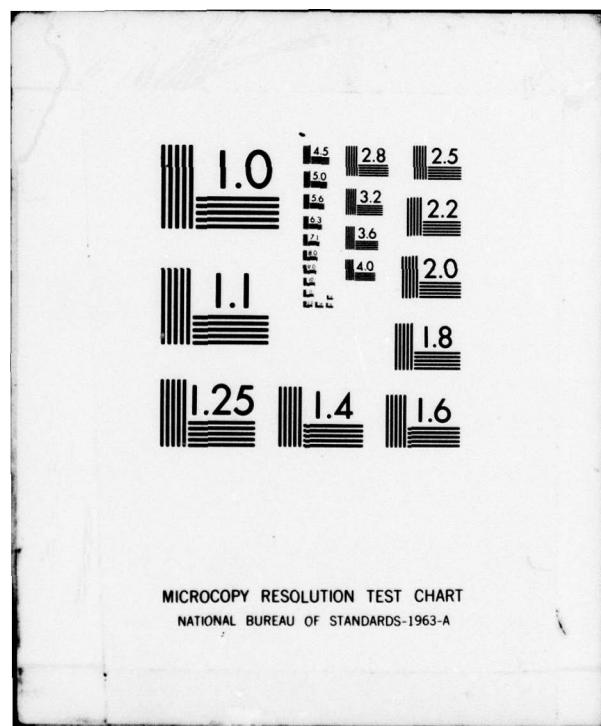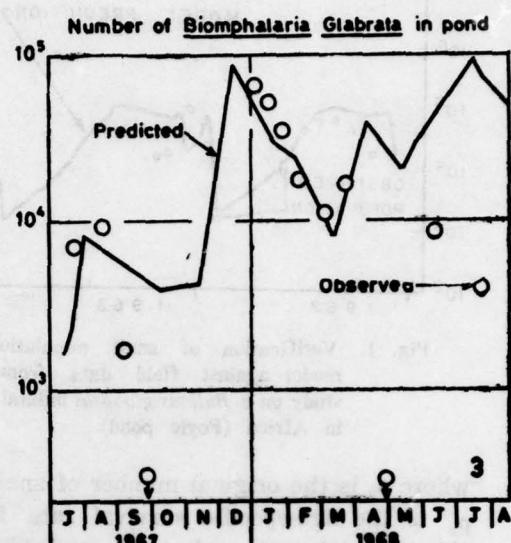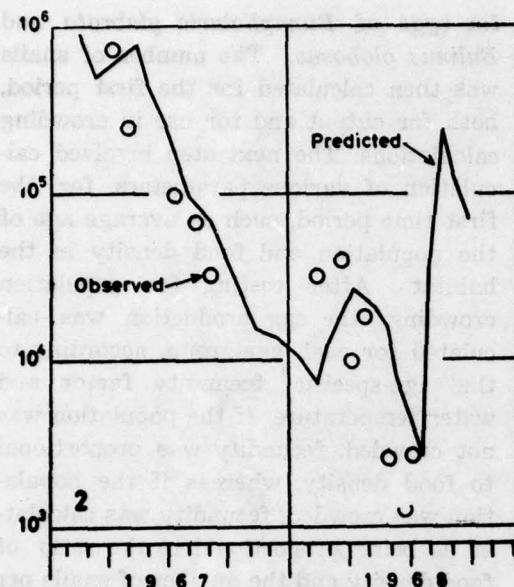
Fig. 2. First verification of snail model for planorbis snails in Puerto Rico; *Biomphalaria glabrata* (Pond B).

**Fig. 3.** Second verification of snail model for planorbids in a Puerto Rican habitat (Pond C).
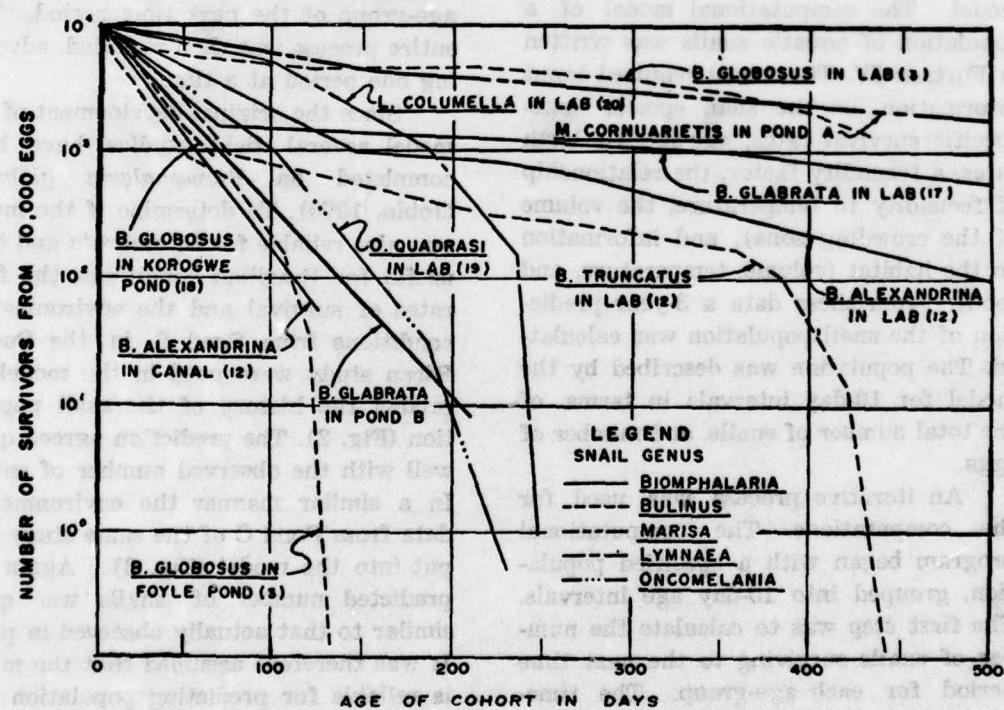
Fig. 4. Age specific death rates for aquatic snails from laboratory and field studies.

Data were obtained on typical reservoirs in the north-east of Brazil near Recife, and in Minas Gerais near Belo Horizonte. The climates are quite different in the two areas, and so are the abilities of the local strains of snails to resist desiccation. Thus the normal population histories in similar reservoirs would be quite different, and the optimum timing of control measures would not be the same for the two areas.

Reservoir in North-East. The natural environmental conditions in a reservoir in the north-east were taken from a description of snail habitats near the city of Recife (Barbosa, 1962). There is a 4-month period from December through March when the habitat is dry and the snails survive by estivation. These snails have a very high resistance to desiccation. The rains begin in April and the reservoir is full of water by May, gradually diminishing in volume from July to November as the rains decrease. The

amount of vegetation increases from April to August and then stays at a constant relative density (kilograms of vegetation per cubic meter of habitat) until the dry season. Water temperatures are always above 25°C and go as high as 35 to 40°C as the habitat dries. This typical climate produces a maximum number of snails in August through October, with the minimum occurring in April or May at the end of the drought (Table 1).

Chemical applications. In order to determine the optimum time of year for application of molluscicides in a reservoir in the north-east, 99% mortalities from chemical treatment were simulated for one month at a time for April, May, July, August, September and October, assuming double applications of molluscicides during a month, spaced 20 days apart. Only 99% mortalities were simulated in order to show the population recovery to be expected following the chemical treatment.

TABLE 1. Model prediction of the population history of *Biomphalaria glabrata* in a reservoir in north-east Brazil, under natural conditions.

Date 1971	Number of snails predicted	Water temperature in °C	Habitat volume in m³	Amount of food in kilograms
January	26,508	40.0	0	0
February	20,307	40.0	0	0
March	15,556	40.0	0	0
April	4,796	28.0	500	10
May	9,613	27.0	9,000	100
June	24,002	26.0	8,000	500
July	43,580	25.0	6,000	1,000
August	113,468	26.0	3,000	1,000
September	122,713	27.0	1,000	1,000
October	152,373	28.0	100	100
November	69,940	35.0	50	50
December	42,331	40.0	0	0

The effect of the molluscicide was most pronounced if the chemical was applied in November or October, just before the onset of the dry period. Only minor decreases in the number of snails occurred if the chemical was applied during the breeding season, primarily because of the high reproductive rate of the snails (Fig. 5). If the applications were made in May, the lowest number of snails during the year was 111, immediately following the first application (Table 2). The next week there was a burst of egg-laying due to the environmental conditions and the uncrowded habitat, thus the number of snails rose to 2,251 and 23,000 eggs were deposited, leaving 182 snails alive after

the second application. At this time the reservoir was full, containing 9,000 cubic meters of water. For the remainder of the breeding season the number of snails increased steadily.

In contrast, the chemicals applied in November caused the snail population to drop to 10 by April (Table 3). There was no recovery of the population following the application of molluscicides since water temperatures were too high for oviposition and the habitat dried-out completely by December, forcing the 48 survivors to estivate (Fig. 5). At the time of the November treatment the reservoir was low, containing only 50 cubic meters of water.
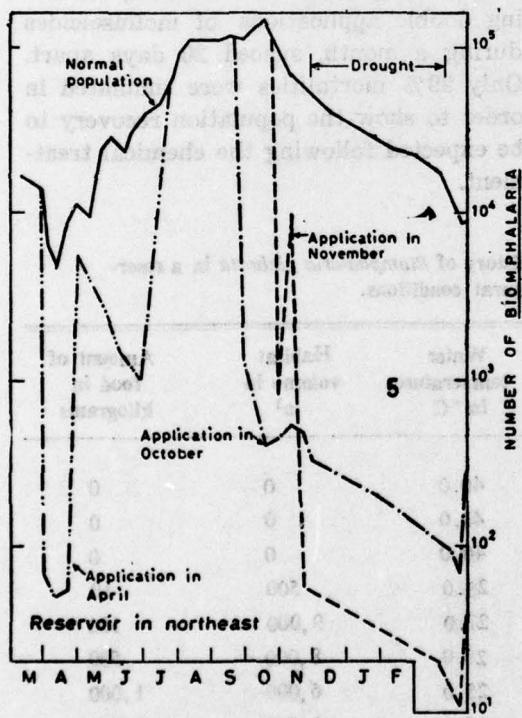

Fig. 5. Prediction of snail populations for reservoir in northeastern Brazil, under natural conditions, and under simulated applications of molluscicides in April, October and November.
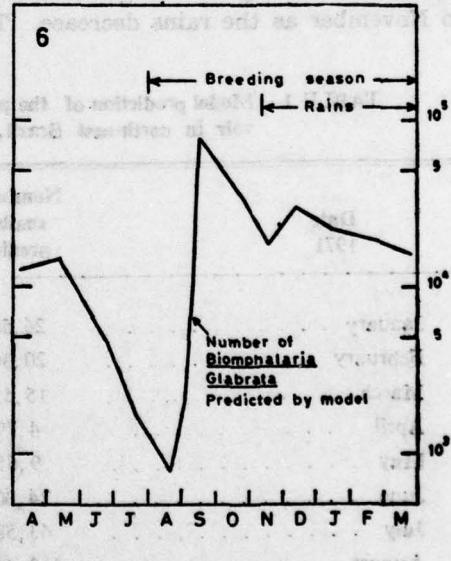
Fig. 6. Simulated snail population for reservoir in Minas Gerais under normal environmental conditions.

TABLE 2. Model prediction of the population history of *Biomphalaria glabrata* in north-east Brazil with double applications of 99% effective molluscicide in May 1971.

Date 1978	Number of snails predicted	Habitat volume in m³
April	4,796	500
	(1st molluscicide application)	
May 1 . . .	111	9,000
May 11 . . .	(2,251	9,000
	(2nd molluscicide application)	
May 21 . . .	182	9,000
June	511	8,000
July	398	6,000
August . .	31,708	3,000
September . .	16,672	1,000

TABLE 3. Model prediction of the population history of *Biomphalaria glabrata* for reservoir in north-east Brazil with double application of 99% effective molluscicide in November 1971.

Date	Number of snails predicted	Habitat volume in m³
1971 October	152,373	100
	(1st application of molluscicide)	
November 1 . . .	855	50
November 11 . . .	9,018	50
	(2nd application of molluscicide)	
November 21 . . .	57	50
December	48	0
1972 January	37	0
February	28	0
March	22	0
April	10	500
May	556	9,000
June	955	8,000
July	16,012	6,000

By tabulating the minimum number of snails predicted following the molluscicide application for each month, it became clear that November ranked first as the month for treatment wihch would cause the snail population to drop to its lowest level, with April, October, May and September following in that order of effectiveness (Table 4). In addition the cost of the application was estimated, based on the habitat volume at the time of treatment and on the available data on chemical and labor costs. From this analysis it was also determined that November was the cheapest month for treatment, followed by October. Combining cost and effectiveness in the analysis by multiplying the minimum number of snails by the cost of treatment, it was determined that the optimum month for treatment was November, followed by October, April, September and May in that order of rank. Although the precise numbers developed in the analysis have little significance, it is clear that the best time to apply molluscicides in the north-east is immediately before the drought, in October and November.

Reservoir in Minas Gerais. The only area in Brazil besides the north-east where a great deal of data is available on the population dynamics of *B. glabrata* is near Belo Horizonte in Minas Gerais. A typical small reservoir with environmental conditions of water temperature, volume and amount of food was simulated for Minas Gerais, using data from Lake Santo and from Lake Pampulha (Fig. 6).

The predicted history of the snail population was similar in general terms to that observed for Lake Santo (Paraense & Santos, 1953), and for Lake Pampulha (Andrade, 1963). Water temperatures fall below 20°C from May through July, restricting the breeding season to the other 9 months of the year (Table 5). Even during the warmer season, mean monthly temperatures of the water do not exceed 25°, being much cooler than the north-east. Although there is no severe dry season in Minas Gerais, the water levels do recede during the cold season, reaching a minimum before the rains start in October. During the hot rainy season (October through April), the heavy rains

TABLE 4. Ranking of months in terms of greatest cost/effectiveness for molluscicide application, for simulated reservoir in north-east Brazil.

Month of application	Minimum number of snails in year following application	Volume of habitat at time of treatment in m^3	Cost per treatment NCr$	Ranking factor snails × cost	Rank
November	10	50	4.22	42	1
April	48	500	6.25	300	3
October	66	100	4.45	294	2
May	111	9,000	56.50	6,300	5
September	123	1,000	20.50	2,520	4

cause the lakes to fill and also produce high levels of turbidity due to the fine silt carried in by the flood waters. This high turbidity restricts the growth of algae and thus retards the amount of food available for snails until about April when the rainfall decreases. During the dry season the water becomes clearer and aquatic growths increase, providing more food for the snails. Under these conditions the model predicted a maximum snail population in September with a minimum in July and August, agreeing in general terms with the observations for lakes in Minas Gerais. The reservoir simulated for Minas Gerais has a maximum volume of 9000 cubic meters, the same as the reservoir simulated for the north-east, which makes treatment costs comparable.

A double application of molluscicides was simulated for each month, assuming the applications occurred on the 1st and 21st days of the month and that each application caused 99% mortality, identical to the treatment regime simulated for the reservoir in the north-east. Under these conditions the snails were completely eliminated if the molluscicide was applied during May, June or July. Since this made it impossible to rank the months comparatively, the mollusciciding was resimulated assuming only 90% mortality.

The fact that the mollusciciding in Minas Gerais had a greater effect on the snail population than did the mollusciciding in the north-east indicates that snail control programs are more likely to succeed in Minas Gerais, due to the nature of the environment. The lower temperatures in Minas Gerais cause a lower average oviposition rate, requiring more time for the snail population to recover from a catastrophe.

For a reservoir in Minas Gerais, the best month for mollusciciding was found to be July, while May and June (the other months when it is too cold for oviposition) are almost as good (Table 6). Chemicals applied during the breeding season have markedly lesser effect on the number of snails (Table 7). In addition to achieving a greater effect when applying molluscicides during the cold months, the cost will be slightly lower, since the reservoirs, although far from dry, will have somewhat less water.

TABLE 5. Model prediction of the population history of *Biomphalaria glabrata* for reservoir in Minas Gerais under natural conditions.

Date		Number of snails predicted	Water temperature in °C	Habitat volume in m³	Amount of food in kilograms
1971	May	14,742	19.0	8,000	200
	June	5,418	18.0	7,000	400
	July	2,060	18.0	6,000	500
	August	745	20.5	5,000	500
	September	79,320	21.0	4,000	500
	October	31,945	21.5	4,000	500
	November	19.590	22.0	9,000	50
	December	29,403	22.5	9,000	50
1972	January	22,663	23.0	9,000	50
	February	19,928	22.0	9,000	50
	March	17,239	21.0	9,000	50
	April	11,467	20.5	9,000	100

TABLE 6. Model prediction of the population history of *Bicmphalaria glabrata* for reservoir in Minas Gerais with double application of 90 % effective molluscicide in July 1971

Date (1971)	Number of snails predicted	Habitat volume in m³
June 11	5,418	7,000
June 21	3,978	7,000
(1st application of molluscicide)		
July 1	289	6,000
July 11	206	6,000
(2nd application of molluscicide)		
July 21	15	6,000
August 1	11	5,000
August 11	7	5,000
August 21	69,496	5,000
September	78,532	4,000

TABLE 7. Model prediction of the population history of *Biomphalaria glabrata* for reservoir in Minas Gerais with double application of 90% effective molluscicide in December 1971.

	Date	Number of snails predicted	Habitat volume in m³
1971	November	19,590	9,000
	(1st application of molluscicide)		
	December 1	3,015	9,000
	December 11	2,940	9,000
	(2nd application of molluscicide)		
	December 21	991	9,000
1972	January	1,229	9,000
	February	2,072	9,000
	June	1,290	7,000
	July	499	6,000
	August	187	5,000
	September	78,880	4,000

A ranking of the various months was made for the reservoir in Minas Gerais, following the previous example. On a cost-effectiveness basis, July ranked first, followed by June and May. The period from May to July is the cold, dry season in Minas Gerais (Table 8). Thus the comparative analysis of the two reservoirs showed that mollusciciding will be more effective in Minas Gerais than in the north-east, primarily because temperatures for reproduction of the snails are more favourable in the north-east. In addition the analysis shows that molluscicides should be applied just before the dry season in the north-east, but during the dry season in Minas Gerais.

The process employed in arriving at these conclusions on the timing of molluscicide operations is a general procedure which can also be applied for other control methods, for other regions of Brazil and for other species of snails. It can also be applied without the use of the computer model, although it then becomes much more time consuming.

Model of Mammalian Host Populations

The mammalian host population in an endemic area is usually the human population, easy to calculate and predict for periods of 10 or 20 years. Of additional interest, however, are rodent populations and other mammals with short life-spans. The rodents have the further value of suitability for laboratory research. Thus the following model was developed as the second main component in the schistosome cycle (Jobin et al., 1969).

A theory was developed to explain the population dynamics of the brown rat based on extensive data on rat ecology and sociology by Calhoun (1962). This «Harem Theory» postulates that the birth rate is regulated through a social organization by limitations of food harborage and population density. The birth rate decrease to zero when the air temperature drops more than 1.5°C per month.

TABLE 8. Ranking of months in terms of greatest cost/effectiveness for molluscicide applications, in simulated reservoir in Minas Gerais.

Month of application	Minimum number of snails in year following application	Volume of habitat at time of treatment in m^3	Cost per treatment in NCr$	Ranking factor snails × cost	Rank
April	90	9,000	56.50	5,100	5
May	7	8,000	52.00	364	3
June	7	7,000	47.50	343	2
July	7	6,000	43.00	300	1
August	75	5,000	38.50	2,810	4
December	187	9,000	56.50	10,000	6

The Harem Theory gave a reasonably accurate estimation of the number of rats observed during a 2-year study of an experimental population. A computer program or population model based on the Harem Theory was used to calculate the number of rats at intervals of 25 days (Fig. 7).
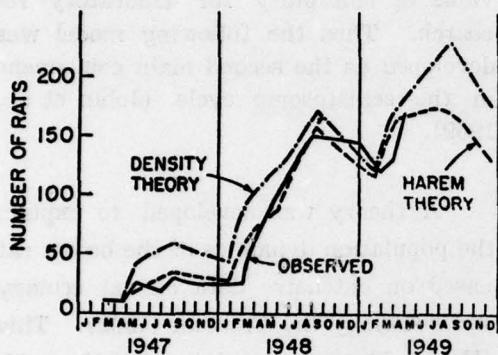

Fig. 7. Population dynamics of brown rat.

A survey of conditions in alleys of Back Bay, Boston, indicated that harborage was the limiting factor for the rat populations, whereas adequate food and excess space were available.

Using the 2-year study on rats to calibrate the computer model, the number of rats in a Back Bay alley was then estimated. The population fluctuated between a maximum of 150 rats in August and a low of 90 rats in February.

Gaps in Knowledge Related to Models

1. The model of the snail population could be improved by developing simpler field methods for determining the amount of food available for the snails. Quantitative sampling of macroscopic vegetation was used in the previous field studies but this is a combersome technique. It should be possible to develop a correlation between the standing crop of vegetation

and measurement of nutrients or photosynthetic productivity.

2. Additional data on oviposition rates and death rates are needed for each snail species, as well as desication survival rates.

In addition, the more complex models of schistosome transmission require further development and verification. They include :

3. Miracidia - sporocyst - cercariae phase as an addition to the snail model. This can be accomplished by subdividing the snail population into non-infected snails and infected snails. For each age group, it will be necessary to compute the number of snails with infections which occurred one month previously, 2 months previously, and so on, up to the age of that snail group. Cercarial production would be a summation of all the snails with patent infection, adjusted by a parasite multiplication factor tied to the age of the infections (Sturrock). The basic data are available although the effect of water temperature must be further delineated.

4. Schistosomule-worm-egg phase as an addition to the rat model. This can be accomplished in a manner similar to the miracidia-cercariae phase. However there is another degree of complexity since the production of eggs is related to the age of the worms and probably to the number of worms and size of host species. The occurrence of new infections superimposed on existing worm population, the relation of egg production of the worms to egg excretion by the mammalian host, the host reaction to infection and the host reaction to diagnostic techniques (fecal examination, skin tests, serological tests, rectal biopsy) should all be included in this model if it is to be eventually verified against human populations.

5. Parasite-host dispersion effects. In order to link the components into a transmission cycle it is necessary to define the dispersion ability, hunting ability, survival in the free-living stages and other characteristic of the parasite. The relevant parameters will be concerned with the geometry of the habitat, distribution of snails and mammalian water contact, water temperature and contamination patterns of the habitat.

6. Stratification effects. To simulate transmission over a large area, there will be several categories of habitats and human populations. A useful model should handle these separately on a habitat basis, finally integrating the several categories in their effect on the human population.

Ecological and Habitat Control Methods for Schistosomiasis Control

There are a small number of control methods under development which can be used to modify the ecology and habitats of the snails which transmit schistosomiasis. These methods are treated here by the categories of irrigation systems, flood control schemes and drainage system which are the main categories of engineering approach for habitat modifications.

Irrigation Systems
Storage reservoirs

Design. There is no proven design which will prevent planorbid snails from colonizing a reservoir. However there are a number of simple techniques which will reduce the extent of snail habitats within a reservoir. These consist of methods which reduce the extent of shoreline suitable for snail habitats. The first and most obvious method is the straightening of the shoreline by earth moving, follow-

ing the general practice in the Tennessee Valley Authority (TVA) reservoirs in the USA which had been developed to reduce mosquito habitats. A second method is the clearing of stumps and vegetation previous to filling the reservoir, thus removing protection for the shoreline and subjecting it to the unreduced action of wind-generated waves (TVA, 1946).

The intake of the discharge conduit should be located in a manner which will minimize the passage of floating debris (including snails). This intake should be located as far as possible from the shore for the same reason. If a minimum pool is retained in the reservoir, provisions should be made to withdraw water from below the minimum pool elevation for as long as possible.

Trash racks to protect the discharge conduit are important and should be designed for easy cleaning. Combined with trash booms to prevent debris from passing over the spillway during flood flows, these devices will minimize the downstream transport of snails and snail eggs. It is recognized that screens small enough to prevent all snails from passing are not practical. However the use of coarse screens which are regularly cleaned and maintained will have a beneficial impact on the reduction of snails transported downstream from the reservoir.

Maintenance. Provisions should be made in the staffing and budgeting of the Authority which owns the reservoirs for regular maintenance of the trash racks and intake structures on the reservoirs, and for removal of aquatic vegetation. The many methods useful for vegetation control have been outlined in the publication by the TVA, including periodic fluctuation of the water level to strand floating vegetation. This stranding should be followed by pilling and

burning of the stranded debris to insure destruction of the snails. In addition the shoreline of the reservoir should be kept free of vegetation as the water level recedes, thus exposing stranded snails to the direct sun and to predators, reducing their chances of survival. In areas where transmission is known to occur, the transmission sites should be fenced to prevent human access, and provisions should be made for reaching the water at other sites where snails are absent. The fences should be kept in good repair and the population periodically informed that they are being prevented from reaching the reservoir because of the danger of schistosomiasis transmission. The fences should contain graphic signs, understandable to children, indicating the danger.

Main transport canals

Design: Perhaps the critical factor in determining whether a large canal will support snail colonies is the velocity of the water at the perimeter of the canal, and the condition of the canal lining. A fair amount of information is available on the velocities required to prevent snails from adhering to a surface, and this information can be used to give design criteria for average velocities in trapezoidal canals which are well maintained.

In a laboratory study with *Biomphalaria glabrata* a velocity of 33 cm/sec. at shell height caused the snails to face into the flow and stop, immobilized by the need to adhere to the surface against the force of the flowing water. At lower velocities the snails migrated against the current. At high velocities, about 65 cm/sec., the snail was dislodged from the surface. Knowing the required velocity conditions at the top of the snail shell, it was possible to calculate the mean velocity for a given channel section which would produce the desired effect (Jobin &

Ippen, 1964). Such mean velocities have been determined for the wide range of channel geometries normally encountered in practice (Table 9). They are significantly higher than values recommended from field observations and thus should prove quite conservative in design.

TABLE 9. **Mean velocities in trapezoidal channels for control of** *Biomphalaria glabrata*.

Canal discharge m³/sec.	Mean velocity for immobilization cm/sec.
1	58
5	67
10	71
20	75
30	78
50	81

Several days of field observations in a small semi circular concrete channel in Puerto Rico showed that the snails migrated upstream if the main stream velocity was below 20 cm/sec., but that they began to move downstream at velocities between 20 cm/sec. and 30 cm/sec. If the velocity went as high as 32 cm/sec. the snails would migrate downstream at a rate of 7 meters per day. Presumably, slightly higher velocities would wash them out (Etges & Frick, 1966). The differences between these field observations and the laboratory study is a reflection of the fact that the laboratory observations lasted about an hour and the snails were able to maintain their position for that short time. However in the field observations, the measurements covered a span of several days and nights, during

which the snails searched for food, were influenced by sunlight, changes in dissolved oxygen, etc. In the field observations the immobilizing velocities were closer to 20 cm/sec. instead of the 33 cm/sec. suggested by the laboratory study. This reinforces the statement that the design values given in Table 2 are conservative and contain a considerable factor of safety.

Studies of *Biomphalaria* distribution in streams in Venezuela have shown their absence when the average velocity of the stream is above 30 cm/sec. (Scorza et al., 1961). Observations in the Belo Horizonte in Brazil showed that the tendency of the snails to migrate against a current leads to repopulation of canals after application of molluscicides (Paulini, 1963). In a ditch where the average water velocity was 10 cm/sec. the snails migrated upstream about 1 meter per day, in agreement with the field observations from Puerto Rico (Etges & Frick, 1966). It was suggested that prevention of upstream migration by the use of velocity «barriers» in canals should be tested experimentally.

Operation. Periodic flushing of the snails from a main canal could wash out the snails instead of using molluscicides. At certain times of the year, if the regular operation of the canal permits it and there are provisions for draining and then flushing the canal, this method should be quite effective. Its use has not been documented, however, and it will not always be practical. It requires that the head gates be capable of rapid opening so that the advancing wave will provide velocities high enough to dislodge the snails. The effect of the wave will be dissipated after a few kilometers, and the snails washed out by the wave must be eliminated.

Maintenance. If the canals are kept free of silt and vegetation they will support fewer snails than if they are not properly maintained. Many of the herbicides used in canals are also lethal to the snails, thus it is recommended that the canals be cleaned regularly if there are snails present. Grammoxone is the prefered herbicide for such applications. If vegetation is removed manually it should be pilled on the banks, dried, and burned.

Distribution canals and equalization reservoirs

Small equalization reservoirs (sometimes called night-storage ponds) are common in sugar irrigation systems and usually have storage capacities of about 5,000 cubic meters. They hold the flow from the main canal for short periods when water is not being applied to the crops. In this way the flow settings upstream do not require daily adjustment, and the size of the delivery canal can be reduced. Unfortunately these ponds often become foci for transmission of schistosomes. They are good snail habitats and are often used for swimming so there is a sufficient amount of fecal contamination and water contact to cause considerable transmission.

The elimination of these ponds will require that water delivery be adjusted more frequently at the main delivery points, and perhaps the delivery canals will have to be designed to carry a larger flow. This additional operating expense will be offset somewhat in areas of high land cost by the area made available for crops when the reservoirs are eliminated. If the reservoirs must be retained in the system, their number should be kept at a minimum and they should be fenced to prevent human contact.

Field drains

In many sugar irrigation projects the main sites of transmission are in the drainage system since conditions there are most favorable for the snails and most of the water contact by people occurs there. Thus the drainage system must be carefully designed, operated and maintained by the usual engineering measures, to minimize the amount of water and vegetation in the ditches. In areas where a great deal of human contact with the ditches would inevitable occur, subsurface drains should be used.

Housing

Housing is often constructed for the farmers in new irrigation schemes, such as the cooperative farms at Bebedouro along the San Francisco River. Proper location of this housing and adequate sanitary facilities can help to minimize transmission of schistosomiasis. The housing should be congregated so that safe water and adequate sewage disposal is economically feasible. In systems where housing is scattered, it is too expensive to supply treated water or sewage disposal for each home.

Housing should not be located near main canals or storage reservoirs in the delivery system, since the labor force must work in the water in the process of irrigating the crops. If the delivery system contains snails and is contaminated by infected people, then the workers will be continually exposed to cercariae-infested waters.

Flood Control and Multi-Purpose Reservoirs

If a reservoir is built strictly for flood control purposes there is seldom any problem with it becoming a snail habitat or transmission site since the

changes in water level are drastic, the water is usually very turbid and the structures are often in inaccessible areas. However if the reservoir is used for both irrigation and hydroelectric power, then it must be treated in much the same way as an irrigation reservoir.

Another type of reservoir is that found on small farms common in areas such as north-eastern Brazil where the water is used for livestock, for human consumption, and for irrigation. Many of these reservoirs were built by DNOCS since 1936, especially in Ceará. Although the method discussed here has not received extensive field testing, it is highly probable that if it were used in these ponds, the snails would be unable to establish colonies.

Small reservoirs or farm ponds which have continual inflow and are full most of the year are usually equipped with an overflow pipe to prevent the *water from rising to the* level of the emergency spillway. If this overflow pipe is capped with a pipe siphon of the same diameter, the water level will drop periodically, stranding some snails and almost all the eggs for short periods. This should drastically reduce the normal reproduction rates in the ponds and eventually eliminate the snail populations.

Requirements for stranding *B. glabrata* on the shores of reservoirs were investigated by studying the behavior of the snails on a submerged slope. The snails migrated down the slope at a speed depending on the slope, the water temperature, the light intensity and the surface roughness (Jobin & Michelson, 1969). For daytime conditions it was estimated that snails can be stranded with a vertical drawdown rate greater than 23 cm/hr on a 5:1 slope and 0.1 cm/hr on a 100:1 slope.

A mathematical model of a snail population was used to evaluate the effectiveness of this technique in two reservoirs, one located in the north-east and one in Minas Gerais (Jobin, 1966). The mathematical model is described in detail in Chapter IX and also in another publication (Jobin & Michelson, 1967). Water-level fluctuations involving cycles with 10 day periods were simulated in hypothetical reservoirs containing 15,000 cubic meters of water. It was determined that the technique would probably not eliminate the snails in the north-east due to temperatures favorable for rapid reproduction and because of the snails high resistance to desiccation (Fig. 8). However in the reservoir in Minas Gerais the technique appeared to have some merit, the prediction being that an initial snail population of 40,000 snails would be reduced to zero within less than 2 years.
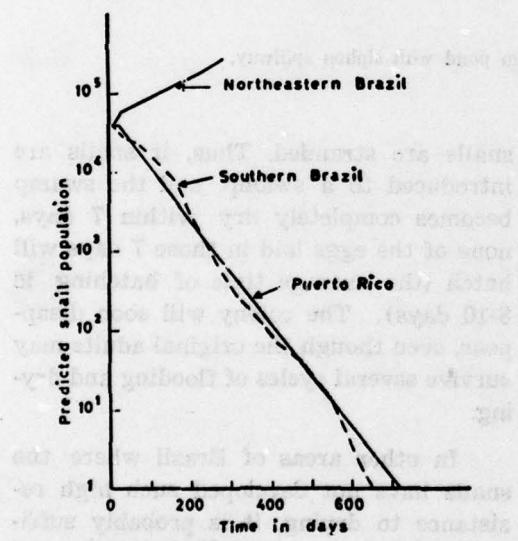
Fig. 8. Projected number of snails in reservoirs with regular schedule of fluctuations in water-level, for conditions of north-east and for conditions of Minas Gerais.

Siphon spillways for snail control were constructed on two small ponds in Puerto Rico (Jobin, 1970). The fluctuations caused by the siphons eliminated the snails from one of the ponds within 4 months after the siphon began to function. The observed drop in water level was 0.5 meters with a fluctuation period of 20 days. The rate of vertical drawdown averaged 3 cm/hr and the shore slope was 13:1, about the rate predicted from laboratory studies. The snails remained absent from the pond for about a year, reappearing only when local inhabitants plugged the siphon to prevent the undesirable water-loss. When the siphon was cleaned out and put into operation, the snails again disappeared.

In the second pond the siphon spillway did not eliminate the snails (Fig. 9). It was designed to produce a somewhat lower drawdown rate and the shore slopes of the reservoir were steeper. However the main reason for its lack of effect appeared to be the low and unreliable inflow to the reservoir. The level in the reservoir rose high enough to cause priming only twice in the first 6 months of operation. With this low frequency of priming and the lower rate of drawdown, the snail population maintained itself at a low level, despite the occasional operation of the siphon.

The use of siphons for fluctuation of the water level in reservoirs should be considered experimental and should be tried only on a pilot scale with careful evaluation of the hydrology and the snail populations. Unfortunately the technique is least likely to succeed in areas where severe droughts have caused the snails to develop considerable resistance to desiccation and where the inflow to such ponds is very intermittent.
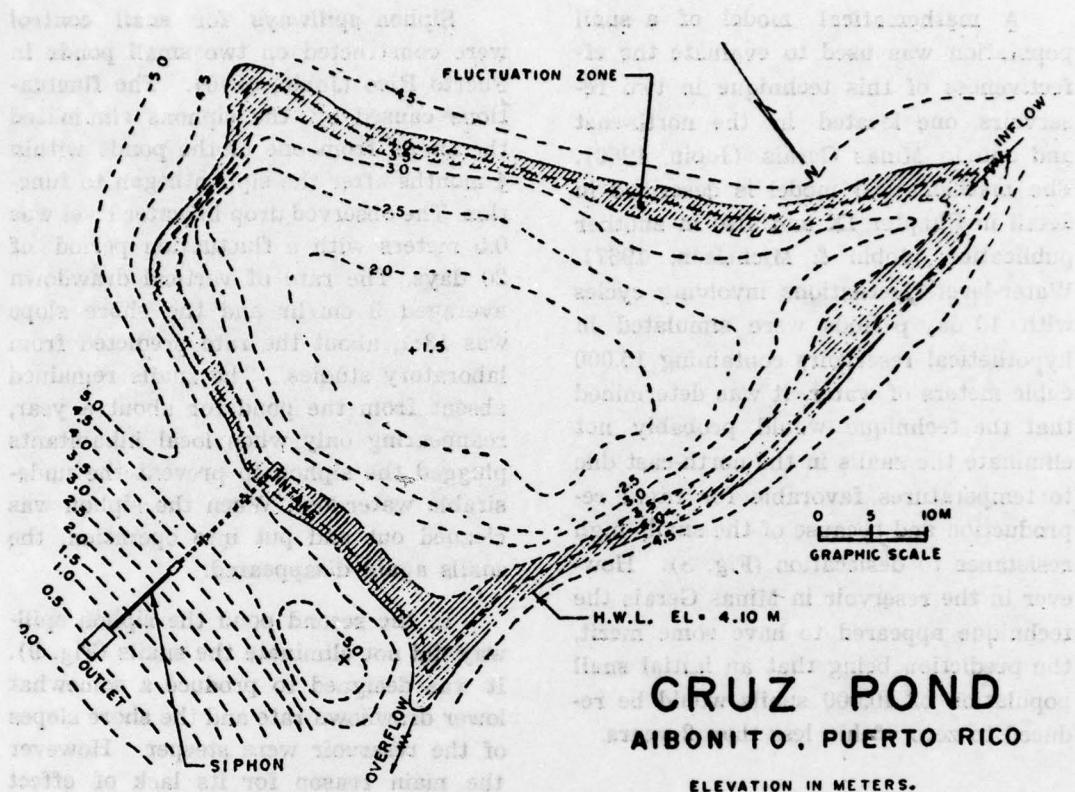
Fig. 9. Topographical plan of farm pond with siphon spillway.

Drainage Works

The criteria for drainage works to control snails are not as stringent as are the criteria for agricultural drainage. The criteria for Brazil are most stringent in arid zones where the snails have become aclimated to periodic drought and where the adult snails can survive almost a year out of water. In these areas, such as north-eastern Brazil, the drainage system should insure that during the breeding seasons (when water temperatures are between 20°C and 30°C) surface waters should not be allowed to stand for more than 1 week, under average rainfall conditions. Desiccation does not kill all of the adult snails but it does kill the snail eggs and prevents oviposition when the

snails are stranded. Thus, if snails are introduced to a swamp, and the swamp becomes completely dry within 7 days, none of the eggs laid in those 7 days will hatch (the average time of hatching is 8-10 days). The colony will soon disappear, even though the original adults may survive several cycles of flooding and drying.

In other areas of Brazil where the snails have not developed such high resistance to drying, it is probably sufficient to insure that the water does not stand for more than 1 month during an average year, to prevent the snail colony from becoming established. These criteria can be used to estimate the discharge needed to design the drainage canal. If

the land is to be used for crops, then additional drainage capacity will be needed, including subsurface drains to control the water-table. If the land is merely to be used for grazing of cattle however, the surface drainage is probably sufficient. The only rainfall data required for such drainage measures is the average weekly or monthly rainfall throughout the breeding season in the area under consideration.

Costs for drainage are low compared to costs for chemical control of the snails, and usually the other benefits from drainage are more than sufficient to pay for the drainage works. One problem with the drainage of swamps is that the snails may survive in the ditches and the disease may continue to be transmitted in the areas at a reduced intensity. It becomes less expensive however, to control the snails with chemicals when the habitat is reduced to the flow in the ditches.

Since drainage ditches can seldom be designed for high velocities and they are almost never lined, maintenance and clearing of the ditches is required in order to eliminate snail colonization. The use of herbicides is recommended as opposed to manual cleaning. Grammoxone is the herbicide of choice since it is toxic to *Biomphalaria* at herbicidal concentrations (Paulini, 1968).

SOME PREDATORS OF *BIOMPHALARIA GLABRATA,*
INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI*
IN GUADELOUPE, FRENCH WEST INDIES[1]

Jean-Pierre Pointier[2] and **André Delplanque**[3]

In the West Indies, the island of Guadeloupe occupies a particular situation. Situated at the meeting point of the two West Indian bows, the Guadeloupe archipelago offers a diversity of environments which is nowhere else encountered in the small Antilles. On «Grande Terre» (exterior bow), a flat dry and calcareous island, most of the aquatic biotopes are constituted by ponds, small lakes, marshes and several temporary ravines. «Basse Terre» or Guadeloupe itself (internal bow), a volcanic and mountainous island with high rainfall, is on the contrary, an area of running waters ; rivers, torrents and numerous channels. The variety of these biotopes explains the variety of the fauna which is found in the freshwaters of Guadeloupe. Thus, among the molluscan fauna, 21 species have been indexed (Pointier, 1974) of which the most common is *Biomphalaria glabrata,* vector of intestinal schistosomiasis. The predators of freshwater molluscs and in particular of *B. glabrata* are various. They belong to almost all zoological groups and we shall present here the most important ones.

Leeches

Among the few species of Hirudinea found in Guadeloupe, one is seemingly a very efficient predator of young molluscs. It probably is *Helobdella punctato-lineata* Moore, 1939, the capacity of which as a predator upon *B. glabrata* has already been signalled by MacAnnally and Moore (1966). This leech (yet to be identified) measures 1-2 cm and feeds exclusively on molluscs. Under laboratory conditions complete breeding cycles have been realised, with the young leeches being fed on young snails of 5 mm diameter. About 60 leeches have been thus bred separately for several months. Eggs appear rapidly on the ventral part of the animal. The eggs are grouped in one single, roundly shaped mass and remain fixed to the leech during the entire incubation period which, at 25°C, may last from 4 to 8 days. After hatching, the juveniles remain attached for about 19 days on the average. They detached only when reaching the adult stage. A new egg mass appears 4-5 days later. Thus the laying cycle seems to average 30 days for the adult. Every

month, one individual gives rise to about 40 leeches. During one monthly cycle and under laboratory conditions every grown animal eats about 35 *B. glabrata* of 5 mm diameter. All tests with the egg masses of *B. glabrata* have shown that the leeches show no predatory activity upon the eggs of these snails, while, in contrast, *B. glabrata* are devoured immediately after hatching. The leeches are liable to decimate snail colonies and the same phenomenon will probably occur in the field. These leeches which can easily be reared under laboratory conditions, might under certain circumstances be used for biological control.

Insects

Among the entomological fauna, 2 species have a predatory activity : *Belostoma boscii* and *Hydrophilus insularis*.

Belostoma boscii

This bug is very common in all aquatic biotopes of Guadeloupe. Its complete life cycle has been realized under laboratory conditions, using snails of different sizes as exclusive food. The layings (about 200 eggs) incubate in 5-8 days. Larval development includes 5 stages and lasts 2 months on the average and can be divided as follows :

1st stage	6,4 days
2nd „	9,4 days
3rd „	12,7 days
4th „	15,8 days
5th „	17,8 days

The life span of the individual is very variable and may often exceed 7 months under laboratory conditions. The adult eats an average of 2 snails of 15-20 mm diameter per day. In the field, *Belostoma* does not feed exclusively on molluscs. They catch anything passing by : tad-

poles, fry, insects, etc. Nevertheless, they probably are not a negligible factor of the regulation of malacological fauna.

Hydrophilus insularis

Only the larval stages of this insect can have a predatory activity upon snails. The adults are strictly herbivorous. There are several larval stages and all can be malacophages.

Crustacea

A great number of crustaces inhabit the fresh water areas in Guadeloupe, mainly Decapoda, Amphipoda and Ostracoda.

Decapoda

Most rivers and torrents of the island contain important populations of shrimps. One of the most common ones, *Macrobrachium faustinum* has a strong predatory activity upon young snails of 2-5 mm diameter, as was detected under laboratory conditions. No predatory activity could be traced either on bigger snails or on eggs masses.

Ostracoda

Several species of ostracods attack and kill young snails (Lo, 1967 ; Sohn & Kornicker, 1972, 1975). On Guadeloupe the most common species, *Chlamydotheca unispinosa,* does not attack the newly hatched snails but it destroys a large quantity of egg masses whenever these are detached from their support. It is likely that the predatory activity of *Chlamydotheca* is very limited in the field.

Other Predators

Among the other zoological groups, a certain number of animals occasionally feed on the malacological fauna. They are :

- Fishes such as certain Mugilidae, very common in the rivers of Guadeloupe,

- Aquatic birds such as *Butorydes virescens,*

- Rodents like rats (*Rattus rattus* and *Rattus norvegicus*) which accumulate the crushed shells in certain places.

The above enumeration of animals which may have an influence upon the malacological fauna is, of course, not exhaustive. On the other hand, caution is necessary as regards any kind of extrapolation of results from the laboratory to the field. In the field, a species is seldom exclusively malacophagous, and it seems difficult to evaluate the real impact of an eventual predator upon the population of molluscs. More elaborate studies on the dynamics of populations might provide complementary data to this subject.

LITERATURE CITED

Lo, C.T., 1967. The inhibiting action of Ostracods on snail cultures. Trans. Amer. microsc. Soc., 86(4): 402-405.

Mac Annally, R.D. & Moore, D.V., 1966. Predation by the leech Helobdella puncto-lineata upon Australorbis glabratus under laboratory conditions. J. Parasit., 52(1): 196-197.

Pointier, J.P., 1974. Faune malacologique dulçaquicole de l'île de la Guadeloupe (Antilles françaises). Bull. Mus. Hist. nat. Paris, 3à Ser. (235) Zool., 159: 905-934.

Sohn, I.G. & Kornicker, L.S., 1972. Predation of schistosomiasis vector snails by Ostracoda (Crustacea). Science, 175: 1258-1259.

Sohn, I.G. & Kornicker, L.S., 1975. Variation in predation behavior of ostracode species on schistosomiasis vector snails. Symposium Univ. Delaware 14-17 August 1972. Pal. Res. Inst. Ithaca, N.Y., 1975: 217-223.

DISPLACEMENT OF *BULINUS TRUNCATUS* BY *MARISA CORNUARIETIS* UNDER SEMI-ENVIRONMENTAL CONDITIONS IN EGYPT[1]

Emile S. Demian[2] and Erian G. Kamel[3]

Introduction

The present report is the third in a series describing various aspects of the population dynamics of the two fresh-water snails, *Bulinus (Isidora) truncatus* (Audouin)[*], the snail host of schistosomiasis haematobia in Egypt, and the neotropical *Marisa cornuarietis* (L.), in outdoor ditches simulating field conditions such as obtained in tertiary branch canals and drains in Egypt (Demian & Kamel, 1972 ; in press). These studies are part of a wider programme devoted to basic ecological studies of *M. cornuarietis* and to its possible utility in the biological control of schistosome-transmitting snails in Egypt (Demian & Ibrahim, 1968/69 ; 1969 ; 1970/71 ; 1972a, b ; 1975).

The South American ampullariid snail *M. cornuarietis* has aroused interest for the past 20 years, since it became known that, after its accidental introduction to the West Indian island of Puerto Rico, it acted as an efficient competitor of *Biomphalaria glabrata,* the snail vector of schistosomiasis mansoni, in the field (Oliver-Gonzàlez et al., 1956 ; Ferguson et al., 1958 ; Oliver-Gonzàlez & Ferguson, 1959 ; Radke et al., 1961 ; Ruiz-Tibén et al., 1969 ; Jobin, 1970). Observations made in the laboratory have shown that

voracious *Marisa* does not only take over the food supply of the vector snails confined with it but also consumes their eggs and actively predates on them, eating out their soft parts (Chernin et al., 1956 ; Michelson & Augustine, 1957 ; Demian & Lutfy, 1964 ; 1965a, b ; 1966).

The objective of the present investigation was to study the effect of *M. cornuarietis* on *B. truncatus* under conditions approximating as closely as possible to those prevailing in the natural environment in Egypt with a view of assessing its potential value as a biological snail control agent. Such a use, if effective, would represent a much easier and cheaper means of control than the chemical control measures now applied.

It is worthy of note that *Marisa* has demonstrated to be refractory to infection with the common animal and human trematode parasites (Penalver, 1956) and thus does not present any health hazards. There are also good indications that *Marisa* does not constitute a serious threat to terresterial crop plants, including rice (Demian & Ibrahim, 1969).

Material and Methods

The present experiments were carried out in a series of earth-lined ditches

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* This snail is simply referred to as B. truncatus in the text.

established in the botanical garden of the Faculty of Science, Ain Shams University, Abbassia, Cairo. Each ditch is 9 m long 120 cm wide at earth level and 50 cm wide at the bottom, holding about 3 cubic metres of water at a depth of 40 cm (Fig. 1). The ditches were supplied with a constant flow of natural, non-treated, silt-laden Nile water and were draining through openings screened with wire mesh to prevent escape of the snails, as described in greater detail by Demian & Kamel (1972). They were equally exposed to sunshine and shade and harboured a similar natural micro- and macroflora as well as an aquatic fauna of insect larvae, tadpoles, etc.

The *Bulinus truncatus* used in this study consisted of selections of specific size groups from among snails collected in some drains in the Giza Governorate near Cairo. *Marisa cornuarietis* were taken from a laboratory stock colony founded with snails originally obtained from Puerto Rico by courtesy of Dr. F.F. Ferguson, former Director of the Puerto Rico Field Station, Tropical Diseases Section, Communicable Diseases Centre, U.S. Public Health Service.

The snail populations in each ditch were sampled in a uniform manner twice a month, by taking a dip with a dip-net (*cf.* Fig. 3 in Demian & Kamel, 1972) at six marked collecting spots, 180 cm apart from one another. After assessment, the snails caught in those six dips were returned alive to their respective sites in the ditch. The number of snails of any population collected by the 12 dips made in one month (= one sample) founded the basis of comparison.

The monthly average maximum and minimum air and water temperature recorded throughout the period of this study are shown in Fig. 2.

Fig. 1. Photograph of an experimental ditch at Ain Shames University, simulating a branch canal or drain.
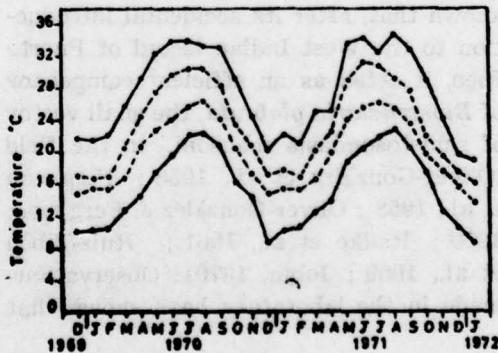

Fig. 2. Monthly average maximum and minimum air temperatures (solid lines) and water temperatures (broken lines) in the experimental area, December 1969 - January 1972. (All averages were calculated from daily maxima and minima).

Experiments

Three experimental populations of *Bulinus truncatus* (B4, B5 and B6), exactly matching control populations in size, numbers and season of nurture, were placed each into an experimental ditch containing a certain population of *Marisa cornuarietis* and were compared with the control populations (B2 and B3) which were kept alone in two other ditches. The latter are the *Bulinus* Populations II and III used in the growth and population dynamics study already reported on (Demian & Kamel, 1972).

The present experiments were set up as follows :

Experiment 1

On April 5, 1970, a cohort of 200 juvenile *B. truncatus* averaging 4 mm in shell length (population B4) was placed in a ditch already containing a group of 125 adult *M. cornuarietis* (Population M2 ; *cf.* Demian & Kamel, in press ; 70 females + 55 males), having a shell diameter of 26-30 mm, which had been placed in the same ditch 10 days earlier (on March 27). The experimental population of *Bulinus* parallelled in every respect a *Bulinus* population (B2) which was established a week later (on April 13), alone, in a separate ditch and which served as control.

The experimental populations of *Bulinus* and *Marisa* were sampled twice monthly starting from May 4, 1970, and the results were compared with those for the control population of *Bulinus* until well after the extinction of the experimental *Bulinus* population in August.

Experiment 2

After the first experimental population of *Bulinus* had died out in August, the experiment was continued by placing into the same ditch, on November 1, 1970, a new cohort of *Bulinus,* with the same population of *Marisa* which at that time, was about four times as numerous as it had been on initial installation in the ditch in March 1970. The bionomics of this *Marisa* population (M2) were under investigation, and are reported on in a separate publication (Demian & Kamel, in press).

The new cohort of *Bulinus* (population B5) consisted of 400 medium-sized snails of 7-8 mm shell length. This population was estimated to correspond in bulk and size to the control population B2, of the first experiment, at that time. The situation thus simulated an optimal state of affairs, i.e. as if, after 7 months of co-existence, *Marisa* had not negatively influenced the preceding *Bulinus* population B4.

Sampling started on November 19, 1970, and comparisons were made until the following April, when the experimental population B5 had plainly disappeared.

Experiment 3

In this third experiment a relatively small group of *Bulinus* (population B6), consisting of 100 adults of 8-9 mm shell length, were added on November 24, 1970, to a population of *Marisa* consisting of 85 snails (population M3 ; 50 females + 35 males) having a shell diameter of 30-32 mm. Another population of *Bulinus* (B3), practically identical with the experimental population, was established in another ditch on the same day as a control.

This experiment was intended to more closely explore the interaction of population dynamics, starting with fewer snails at a season when the activity of

Marisa is curtailed, as in experiment 2, and following up observations for a longer time after the extinction of the experimental population of *Bulinus*. The smaller number of *Marisa* in particular was meant to retard the early overcrowding caused by the vigorous multiplication of this snail.

Starting from December 10, 1970, fortnightly samplings were compared until well after the virtual elimination of the experimental population of *Bulinus* 8 months later, in August 1971.

Results

Experiment 1

A comparison of the experimental and control populations of *B. truncatus* (B2 and B4 respectively) is given, month by month as regards the numbers of snails sampled, in Fig. 3. The numbers of *M. cornuarietis* sampled monthly from the experimental ditch are shown in the same figure, though at a smaller scale.

The data summarized in the left portion of that figure show that for the first 2 months of the experiment (up to June 1970) there were no marked differences in population density between the experimental and control populations of *Bulinus*. However, from then onward, as the *Marisa* population was soaring, the upward trend of the experimental population of *Bulinus* was reversed and its decline became obvious. The number of *Bulinus* dipped out from the experimental population was but a quarter of that obtained from the control population. This decline became further aggravated in July and August. While the control population B2 was still building up steadily, until it reached a peak density in August, the experimental population B4 had dwindled almost to the point of complete elimination. During this same period the population of *Marisa* had been

building up steadily, the monthly samplings being five times as large (from 82 *Marisa* caught in May to 435 in August).

The sample taken from the experimental population B4 on August 4 was the last one that included some live *Bulinus*. None of the seven subsequent samplings, made during the period August 19-October 19, included a single live specimen ; only empty shells of *Bulinus* were found. Thus *Bulinus* had disappeared from the experimental ditch within 5 months, nor did it reappear during the following 2 months, whereupon the experiment was terminated. It was noted that irreversible decline in this *Bulinus* population apparently occurred when the density of *Marisa* exceeded a sample strength of about 200 *Marisa* (in 12 dips).

Experiment 2

The numbers of *B. truncatus* collected every month from the experimental population B5 and from the control population B2 during the period November 1970-April 1971 are also shown in Fig. 3 (right half). For the first 4 months (up to February 1971) changes in density in the experimental and control populations of *Bulinus* were more or less parallel. The *Bulinus* of the experimental population B5 started to oviposit late in November and thus produced a new brood, or «winter» generation, comparable to that which was simultaneously produced in the control population B2. The newly hatched young of those «winter» generations started to appear in the mesh of our nets in the January 1971 samplings from both populations.

During the cold winter months the tropical *Marisa* was noted to be rather sluggish and inactive. Their growth and reproduction practically stopped between

November 1970 and February 1971, while mortality due to gross overcrowding had been reducing their numbers from September onwards, the monthly samplings having steadily decreased from 442 snails in September to around 140 in the months of January, February and March. During that period, the monthly average minimal water temperatures had dropped from 24.2°C in September 1970 to 15.2°C in February 1971, and the maximal water temperatures from 28.5°C to 16.8°C (Fig. 2).

However, from March 1971 onward, as, with increasing temperatures, *Marisa* resumed its normal activities, striking differences between the experimental and control populations of *Bulinus* became manifest. While the experimental population B5 sharply declined, the control population B2 continued its normal seasonal upward trend as a result of intensive breeding and the consequent increase

in number of young snails, the monthly samplings having steadily risen from 26 *Bulinus* in December 1970 to 194 in April 1971. Breeding was far less in evidence in the experimental population and the number of young and adults caught decreased progressively until, in the second sampling made in April 1971, no live Bulinus was collected. The experimental population of *Bulinus* had plainly been destroyed by April, i.e. in about 6 months. This may also indicate that *Marisa*, when active, can at densities of 140-150 snails per monthly sample rapidly exterminate *Bulinus*.

Experiment 3

The number of *B. truncatus* sampled monthly from the experimental population B6 and from the control population B3 as well as that from the *M. cornuarietis* population M3 are show in Fig. 4.

Fig. 3. Numbers of *Bulinus truncatus* sampled each month in 12 dips from control population B2 (kept alone) and experimental populations B4 and B5 (kept together with *Marisa cornuarietis*), and numbers of *Marisa* (population M2) similarly sampled each month.
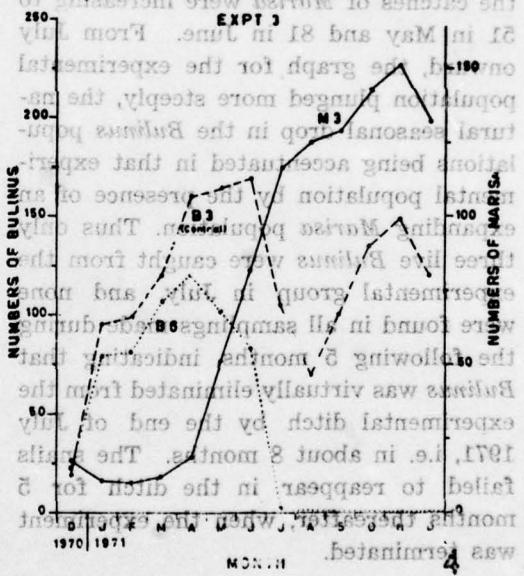

Fig. 4. Numbers of *Bulinus truncatus* sampled each month from control population B3 (kept alone) and experimental population B6 (kept together with *Marisa cornuarietis*), and numbers of *Marisa* (population M3) similarly sampled each month.

Proc. Int. Conf. Schisto. (1975)

The results indicate that the experimental and control populations of *Bulinus* did not significantly deviate in density during the first 4 months of the experiment (December 1970-March 1971) while *Marisa* was practically dormant. Both *Bulinus* populations were building up steadily during those 4 months, although the experimental population was lagging a little behind. At the same time not only were the activities of *Marisa* severely curtailed by the the relatively low water temperatures prevailing during that period, but their numbers were low, the monthly samplings varying between 12 and 18 snails.

From April 1971 onward, after *Marisa* had resumed its normal activities, the experimental population of *Bulinus* decreased quite markedly for the first 2 months (May and June), while the controls were still increasing in number and the catches of *Marisa* were increasing to 51 in May and 81 in June. From July onward, the graph for the experimental population plunged more steeply, the natural seasonal drop in the *Bulinus* populations being accentuated in that experimental population by the presence of an expanding *Marisa* population. Thus only three live *Bulinus* were caught from the experimental group in July, and none were found in all samplings made during the following 5 months, indicating that *Bulinus* was virtually eliminated from the experimental ditch by the end of July 1971, i.e. in about 8 months. The snails failed to reappear in the ditch for 5 months thereafter, when the experiment was terminated.

During that time the controls, after a normal seasonal decline at the height of summer due to excessive heat, were able to re-establish themselves by producing a new «autumn» generation.

It was noticed in this experiment that the presence of *Marisa* at densities of around 100 per monthly sample was still operative in depressing the experimental *Bulinus* population to the point of no return.

Conclusions and Discussion

The principal importance of the results of the present experiments lies in their practical implications with regard to the control of *Bulinus truncatus* in Egypt. They undoubtedly provide encouraging indications of the possible utility of *Marisa cornuarietis* as a biological control agent against that noxious schistosome-transmitting snail.

The significant reduction in density of the three experimental populations of *Bulinus* examined, as compared to the control populations, after an initial period of 2-4 months, and their ultimate disappearance from the experimental ditches in 5-8 months, depending on season and on the relative densities of the populations of the two competing species, illustrate a remarkable ability on the part of *M. cornuarietis* to eliminate and replace *B. truncatus* under the semi-environmental conditions of the present study.

The regular swift decline in the experimental populations of *Bulinus* at the very time when the controls were building up population peaks, and their final extinction when the controls, as well as *Marisa*, were thriving, is striking. Since initially the experimental and control populations of *Bulinus* were closely similar, the observed effects on *Bulinus* cannot possibly be attributed to some environmental or climatic factors or to any factor other than the presence of *Marisa*.

Analyzing the graphs shown in Figs. 3 and 4, one observes that the experi-

mental *Bulinus* populations B4, B5 and B6 for a short while closely parallel their controls, which are supposed to follow the natural seasonal trends, lagging behind only insignificantly. At the same time one notes that the curves for the control populations B2 and B3 do not correspond to one another, the curve for population B2 showing a single annual density peak in the late summer of 1970 (August) and a trough in winter (December), while that for population B3 showed two annual peaks, one in early summer (June 1971) and one in late autumn (November 1971) with an intervening low density in August, although the all important ecological, in particular climatic, conditions were practically the same in 1970 and 1971. The explanation for such a seeming contradiction must be sought in the interplay, with climatic conditions, of the size, i.e. numbers as well as ages, of the experimental groups and of the starting season. These factors influence population structure, i.e. the varying rates of population build-up as well as the speed of overcrowding the available space, and thus the rhythms of waxing and waning. Therefore, when starting with a relatively small group of adult *Bulinus* (100 snails with a shell length of 8-9 mm ; control population B3) in winter, critical density (corresponding to monthly catches of over 150 *Bulinus* in 12 dips) built up by the following June, i.e. in 7 months. From then on many of the old snails succumbed producing a relative low density in August. This is in good agreement with the well known decline of old snails in the natural environment at that time. This old population, including some young snails emerging from it in spring, did however successfully establish a second new «autumn» generation, thus attaining a minor density peak in November of the same year. As for the control population B2, start-

ing out with younger snails (4 mm in shell length) in late spring, equivalent densities could build up in 4 months, with a relatively late population peak in August (when population B3 was at ebb). Those younger snails could tolerate high summer temperatures better than did older snails. Thus, overpopulation and mortality set in later in population B2. The subsequent decline was also more prolonged and reached into winter, precluding the build up of a new generation in autumn of the same year.

It is further noted that the experimental *Bulinus* populations B5 and B6, used in the experiments starting in November 1970, followed the natural trends much longer before they sharply diverged, i.e. for 3-4 months, as compared to barely 2 months for population B4 starting in April. This is without doubt due to the fact that *Marisa* is not very active for most of the winter period, from December to February. Thus population B4 disappeared faster than either population B5 or B6 because there was no initial delay due to inactivity of *Marisa*. Once *Marisa* is normally active and sufficiently numerous the declines in the *Bulinus* populations observed was equally rapid, taking 2-3 months until total disappearance.

The present findings corroborate, for conditions more closely approximating those in the natural environment, earlier reports by Demian & Lutfy (1965a ; 1966) on the capacity of *Marisa cornuarietis* to destroy *Bulinus truncatus* populations under laboratory conditions. Those authors have already provided conclusive evidence that *Marisa* in the laboratory attacks and preys upon *Bulinus*, actively ingests its young and purposefully consumes its egg masses. Confirmation of such predatory behaviour in the experimental ditches used in the present study was not feasible. The details of inter-

actions between these two competing
species could not be as closely observed
in the ditches as in the laboratory aqua-
ria. Thus whether the observed effects of
Marisa on the experimental populations
of *Bulinus* was due to direct predation or
to competition for food and space, or to
a combination of both factors, could not
be determined from the present study.

However, since the prognosis for the
establishment and maintenance of the
tropical *Marisa cornuarietis* under the
subtropical conditions prevailing in
Egypt is good (Demian & Kamel, in
press), the present findings point to the
role *Marisa* could play as an efficient po-
tential competitor to *Bulinus truncatus* in
Egypt, similar to that played against
Biomphalaria glabrata in Puerto Rico.

Should the observations made by
Demian & Lutfy (1965a ; 1966) in the
laboratory and the results obtained
through the present series of semi-envi-
ronmental experiments prove broadly
valid in larger streams and in fluctuating
water bodies in nature, *M. cornuarietis*
could be of great value in the control of
natural populations of *B. truncatus* in
Egypt. The present study would then con-
stitute an important step forward to-
wards the solution of the so far unsoluble
snail control problems in Egypt on a per-
manent and economically feasible basis.

LITERATURE CITED

Chernin, E., Michelson, E.H. & Augustine, D.L.,
  1956. Studies on the biological control of schis-
  tosome-bearing snails. I. The control of Austra-
  lorbis glabratus populations by the snail, Marisa
  cornuarietis, under laboratory conditions. Amer.
  J. trop. Med. Hyg., 5: 297-307.

Demian, E.S. & Ibrahim, A.M., 1963/69. Tolerance
  of the snail Marisa cornuarietis (L.) to desicca-
  tion and to continuous submersion, under labo-
  ratory conditions. Bull. zool. Soc. Egypt, 22:
  73-88.

Demian, E.S. & Ibrahim, A.M., 1969. Feeding ac-
  tivities of the snail Marisa cornuarietis (L.)
  under laboratory conditions. Proc. Sixth Arab
  Sci. Congr., Damascus 1969, Part I: 145-165.

Demian, E.S. & Ibrahim, A.M., 1970/71. The egg
  mass, egg laying and mating behaviour of the
  snail Marisa cornuarietis (L.). Bull. zool. Soc.
  Egypt, 23: 1-12.

Demian, E.S. & Ibrahim, A.M., 1972a. Tolerance
  of the snail Marisa cornuarietis (L.) to extreme
  temperatures under laboratory conditions. Ibid.,
  24: 20-30.

Demian, E.S. & Ibrahim, A.M., 1972b. Sexual
  dimorphism and sex ratio in the snail Marisa
  cornuarietis (L.). Bull. zool. Soc. Egypt, 24:
  52-63.

Demian, E.S. & Ibrahim, A.M., 1975. The repro-
  duction biology of the snail Marisa cornuarietis
  (L.) under laboratory conditions. First Seminar
  on Environmental Research, Ain Shams Univ.,
  Cairo, Nov. 1974. Ain Shams Sci. Bull. 19: 111-
  120.

Demian, E.S. & Kamel, E.G., 1972. Growth and
  population dynamics of Bulinus truncatus under
  semi-field conditions in Egypt. Proc. Egypt.
  Acad. Sci., 25: 37-60.

Demian, E.S. & Kamel, E.G., In press. Growth
  and population dynamics of the neotropical snail
  Marisa cornuarietis under semi-field conditions
  in Egypt. Bull. zool. Soc. Egypt.

Demian, E.S. & Lutfy, R.G., 1964. Prospects of
  the use of Marisa cornuarietis in the biological
  control of Limnaea caillaudi in the U.A.A. Proc.
  Egypt. Acad. Sci., 18: 46-50, Pls. I-III.

Demian, E.S. & Lutfy, R.G., 1965a. Predatory acti-
  vity of Marisa cornuarietis against Bulinus
  (Bulinus) truncatus, the transmitter of urinary
  schistosomiasis. Ann. trop. Med. Parasit., 59:
  331-336.

Demian, E.S. & Lutfy, R.G., 1965b. Predatory acti-
  vity of Marisa cornuarietis against Biompha-
  laria alexandrina under laboratory conditions.
  Ibid., 59: 337-339.

Demian, E.S. & Lutfy, R.G., 1966. Factors affecting the predation of Marisa cornuarietis on Bulinus (B.) truncatus, Biomphalaria alexandrina and Limnaea caillaudi. Oikos, 17: 212-230.

Ferguson, F.F., Oliver-Gonzàlez, J. & Palmer, J.R., 1958. Potential for biological control of Australorbis glabratus, the intermediate host of Puerto Rican schistosomiasis. Amer. J. trop. Med. Hyg., 7: 491-493.

Jobin, W.R., 1970. Population dynamics of aquatic snails in three farm ponds of Puerto Rico. Ibid., 19: 1038-1048.

Michelson, E.H. & Augustine, D.L., 1957. Studies on the biological control of schistosome-bearing snails. IV. The control of Biomphalaria pfeifferi populations by the snail, Marisa cornuarietis, under laboratory conditions. J. Parasit., 43: 135.

Oliver-Gonzàlez, J., Bauman, P.M. & Benenson, A.S., 1956. Effect of the snail Marisa cornuarietis on Australorbis glabratus in natural bodies of water in Puerto Rico. Amer. J. trop. Med. Hyg., 5: 290-296.

Oliver-Gonzàlez, J. & Ferguson, F.F., 1959. Probable biological control of schistosomiasis mansoni in a Puerto Rican watershed. Ibid., 8: 56-59.

Penalver, L.M., 1956. Infection experimental de molluscos fluviale Cubanoc con Schistosoma mansoni. Arch. Venez. Patol. Trop. Parasit. Med., 2: 297-308.

Radke, M.G., Ritchie, L.S. & Ferguson, F.F., 1961. Demonstrated control of Australorbis glabratus by Marisa cornuarietis under field conditions in Puerto Rico. Amer. J. trop. Med. Hyg., 10: 370-373.

Ruiz-Tibén, E., Palmer, J.R. & Ferguson, F.F., 1969. Biological control of Biomphalaria glabrata by Marisa cornuarietis in irrigation ponds in Puerto Rico. Bull. Wld Hlth Org., 41: 329-333.

EVALUATION OF COSTS AND BENEFITS OF HABITAT MODIFICATION USED IN THE CONTROL OF THE INTERMEDIATE HOSTS OF SCHISTOSOMIASIS

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Habitat modification, through drainage and land reclamation with the use of heavy equipment, has often been shown to be the most effective means for eliminating the snail hosts of schistosomiasis, especially for large swampy type habitats. The high initial capital investment which is necessary often discourages project officials from proceeding with this type of work. However, considering the indirect benefits that can accrue in terms of the increase in agricultural productivity of the reclaimed land, added justification to making the initial high capital investment is possible. Ansari (1973), states that radical environmental changes must be evaluated in light of **both direct** and **indirect** benefits, since the agricultural benefits from the reclaimed land in some cases may be enough to completely offset the costs of such work. By emphasizing these indirect benefits, as well as the direct health benefits, the project administrator may have more success in obtaining funds to undertake the work, or in obtaining cooperation for such work from other agencies concerned with general land reclamation and utilization.

Recent habitat modification work undertaken in the endemic area of Khuzistan, Iran, resulted in the elimination of several extensive infested swamps, and at the same time converted previously unusable swamp land into potentially productive farm land. In the following analysis, the costs of this work is evaluated in light of the potential agricultural benefits obtainable, to demonstrate the extent to which these indirect benefits can offset the costs of such work. The land reclaimed in this work has not as yet been put into local production so that an evaluation of actual benefits was not possible. However, with proper inducements and future follow up action by the control project, these economic benefits can be realistically achieved by the local farmers.

Habitat modification input/output relationships

The relationship of the inputs and outputs associated with this work is set forth in Fig. 1. The inputs of equipment, labor, and engineering, planning and supervision can be quantified in both physical and economic terms. The primary output (primary from the point of view of schistosomiasis control), elimination

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INPUTS   OUTPUTS
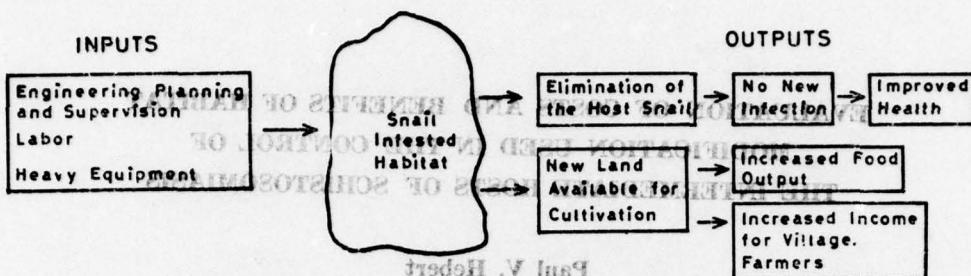
Fig. 1. Input/output diagram for habitat modification in a swampy, snail infested habitat.

of the host snail and subsequent project-ed reduction in disease prevalence, may be quantifiable, but the economic benefit, in most cases, has been shown to be quite difficult to establish. The secondary out-put, the new land made available for cul-tivation and subsequent economic value of the land, is readily quantifiable, and it is this secondary output, or indirect benefit, in light of the input costs, that is of concern in this analysis.

Description of the area of the habitats

Three swampy habitats, 4.5, 5.5, and 14.5 hectares in area, where reclamation work was carried out, were included in the analysis. All three habitats were located in a traditional irrigated farming area, about 25 km south of the city of Dezful, Khuzistan, Iran. The area, about 5000 hectares, contains 15 villages with a total population of about 3000. The area is surrounded by the Dez Irrigation Pro-ject on two sides, and by the Haft Tapeh Sugar Plantation on another. The vil-lages receive irrigation water by means of a privately operated dirt canal system fed by the nearby Abjirob River. Tradi-tional agricultural methods are generally practiced, although recently, tractors and some other modern equipment have begun to be used. The rate of infection of schis-tosomiasis was higher in this region than in others due to high infestation of canals,

drains, ponds, and swamps with the host snail, and frequent contact of the vil-lagers with the habitats. The presence of the swamps were in one way or another related to inadequacies of the irrigation system and in methods of irrigation, as well as to the physical features of the land. They had proven to be too exten-sive for a mollusciciding programme to handle effectively. The swamps were thought to play both a direct and indirect role in the transmission of the disease, directly through contact of the villagers with the water and indirectly as a reser-voir for the host snail. Short of renova-tion of the entire irrigation system and drastically changing irrigation and farm-ing practices, it was decided that the im-provement of local drainage combined with filling and leveling of the low swampy areas would be the most feasible means ot eliminate the host snail, while at the same time providing additional land for cultivation.

Description of work

The engineering work was carried out in the winter and spring of 1973 with funds provided by the Ministry of Health, and included the following :

1. Surveys were made for drainage of the swamps.

2. Drains were constructed and swamps drained as completely as possible.

3. Swamps were surveyed and estimates for contract work were made.

4. Swamps were filled and the land was leveled with the use of contracted heavy equipment.

5. Final drains were then constructed.

Survey work, planning and engineering supervision were carried out by project personnel. The main drains were constructed by hand labor, and field drains were constructed with a grader. The swamps were filled and the land leveled by contract equipment; in Area 1 by 2 bulldozers, and in Areas 2 and 3 by self-propelled scrapers and bulldozers. Table 1 describes the work performed and the labor and equipment used.

Costs of drainage and land reclamation

For each of the three areas, the total costs of drainage work, land reclamation, leveling and yearly maintenance of the drainage system were computed. The cost of engineering, planning and survey work were not figured into this analysis as they comprise mainly salary and transportation costs which can be considered a fixed part of the overall project budget. Labor and equipment rates were calculated at current 1975 rates. These costs data are summarized in Table 2. Initial investment costs were amortized to show the costs on an annual basis so as to be comparable with the annual benefit projections. The cost per m³ of drainage did not vary among the three areas, whereas the cost per hectare of land reclaimed and per m³ of fill material moved varied considerably, particularly between Area 1 and Areas 2 and 3. This is explained primarily because of the difference in equipment used. In areas 2 and 3, large self-propelled rubber-tired scrapers were used to haul fill material, which when used in combination with bulldozers, proved much more efficient than operating with bull-

dozers alone, as was done in Area 1. The total investment costs per hectare, including drainage for areas 1, 2 and 3 were $ 672, $ 438 and $ 396, respectively.

Indirect benefits

The indirect benefits resulting from the reclaimed land were projected as the net increase in agricultural potential of the land. Specifically, these potential benefits were calculated for each of the three reclaimed areas based on the value of crops that could feasibly be grown on the land. Potential yields were based on local data and projected crop income was based on current local average market values. The first two crops considered, wheat and rice, were assumed to be farmed in the traditional manner of the area, but the other crop chosen for analysis, alfalfa, was assumed to require some mechanization, which with some technical assistance, was thought to be within the capabilities of the local farmers.

Although the analysis is limited to these three crops, for overall comparative purposes, data for several other crops suitable for this area were developed. As shown in Table 3, yields can vary considerably depending on the use of traditional or modernized methods of farming. The table also shows that the gross income per hectare for wheat, rice, and alfalfa were projected to be $ 211, $ 590 and $ 1030 respectively, which shows a wide variation in potential gross income. In order to obtain net benefits, annual production costs for each of the three crops were projected and the net incomes per hectare calculated. This left a net of $ 127, $ 362 and $ 767 for wheat, rice, and alfalfa, respectively. Annual production labor costs were not calculated as all labor was assumed to be performed by the villages in the area. The decision not to include labor costs was made for several reasons. First, it is difficult to place a

TABLE 1. Equipment and specifications for land reclamation work.

Area	Equipment	Maximum Haul Distance for Fill Material	Earth Moved		Swamp Filled (hect)	Land Levelled (hect)	Drain Constructed	
			m³/hect.	Total m³			Hand Labor	Grader
Area 1	1 D-6 Bulldozer 1 D-7 Bulldozer	200 m	1080	9700	4.5	9.0	300 m (450 m³)	0
Area 2	1 D-8 1 D-7 2 Scrapers 1 grader	500 m	1640	23000	5.5	14.0	1300 m (1950 m³)	2700 m (1512 m³)
Area 3	1 D-8 1 D-7 2 Scrapers 1 Grader	400 m	1260	41600	14.5	33.0	700 m (1575 m³)	5800 m (4688 m³)

TABLE 2. Costs of habitat modification work.

Area	Drainage costs $/m³*		Land Reclamation**		Total		Total Investment	Maint. of Drains***	Amortized Capital	Average Annual
	Grader	Labor	$/m³	Labor	$/hect.	$/hr.	Total $	$/year	Costs $ §	Costs $
Area 1	—	0.74	0.59	0.74	672	632	6056	66	617	683
Area 2	0.10	0.74	0.21	0.74	458	338	6424	761	654	1415
Area 3	0.10	0.74	0.28	0.74	396	353	13083	938	1333	2271

* Based on the average size of drains and 2.4 m³/day/laborer wage = $ 1.76/day   Grader rental = $ 18/hr.

** Based on hourly rates for equipment
D-6 = $11.76
D-7 = $16.18
D-8 = $20.58
Scraper = $20.58

*** Based on $0.22/ m³ hand labor.
§ Amortized at 8% over 20 years

TABLE 3. Projected agricultural production and expected income from reclaimed land.

Crops	Expected Yields (metric tons/hect.)		Labor Intensive	Average Market Value ($/kg)	Gross Income ($/hect.)	Production Costs ($/hect.)	Net Income ($/hect.)
	Traditional	Modernized (Intensive)					
Wheat	18	3.5	No	0.12	211	84	127
Rice	2.0	—	yes	0.30	590	228	362
Alfalfa . . .	—	7	yes	0.15	1030	262	767
The Crops below are for comparative purposes							
Barley . . .	<1	2.4	no	0.09	90	—	—
Sesame . . .	0.9	1.0	yes	?	—	—	—
Broad Beans . .	2.0	2.4	yes	0.30	600	—	—
Onions . . .	40	—	yes+	0.08— .15	3600	—	—
Field Corn . .	—	4.0	no	.13— .15	765	—	—

Large labor force is required which limits cultivation to small plots.

value on this labor, and presently most of these villagers do not have alternatives for full time outside employment. Also in this case, we are concerned primarily with the net increase in village farm income as the measure of benefits. We can say that if village labor is in short supply, the choice of a less labor intensive crop, such as wheat, might be necessary in lieu of a more labor intensive one, such as rice, or alfalfa, even though the latter may be more profitably grown.

Costs and benefits compared

The costs of the reclamation work were compared against the potential benefits for each area. In the final analysis, because of physical constraints and soil conditions, rice was not considered a viable alternative for Area 3 and wheat was not a viable choice for Area 2. Figure 2 compares the costs and the benefits for the remaining choices. Under all cropping patterns for each of the three areas, annual benefits exceeded the costs of the land reclamation, clearly demonstrating that for these three areas, the work can be justified economically solely on the grounds of the potential agricultural benefits to be gained. The benefits minus the costs did vary considerably from only $ 468 for cultivation of wheat in Area 1 to $ 22969 for cultivation of alfalfa in Area 3.

Factors affecting costs and benefits projections

Many factors exist which affect both costs and indirect agricultural benefits. Generally, the factors which most greatly affect the costs of this type of work from area to area have been found to be :

1. The availability of the proper type of heavy equipment.

2. The amount of drainage work necessary.

3. The availability of fill material.

4. The accessibility of the area to heavy equipment.

Conditions regarding these factors will greatly affect the per hectar cost of reclamation work and must be carefully considered when making cost estimates. In some areas, improvement of drainage alone might be the only engineering activity necessary to dry up a swampy area permanently, making additional land available for cultivation. In such a case the costs of reclamation would be greatly lowered.

Generally, the estimates of economic benefits are quite complicated. The estimates require an intimate knowledge of (1) the local areas considered for reclamation, (2) the soils of the area, (3) traditional agricultural technology and capabilities for modernization, and (4) the potential of the land for supporting specific crops. Such estimates would best be made with the assistance of local agricultural experts if such help is available. If care is taken by the engineer in making these estimates, a very reliable picture of the economic feasibility can be gained for performing this type of work.

Other considerations

Certain other considerations must not be overlooked when proposing such work, or in selecting the means for realizing the hoped for benefits. For example, in many areas where schistosomiasis is endemic, an association of the disease with rice production has been found due to standing water in the fields for long periods of time. Therefore, one might be hesitant to consider rice as a potential crop. With proper water management, however, this problem can be controlled. Also, introducing a modern crop such as alfalfa, where traditional agriculture is practiced, may not prove successful if new technology and training of local farmers is not introduced at the same time.

AVERAGE ANNUAL COSTS $		ANNUAL BENEFITS $	BENEFITS MINUS COSTS $
	AREA 1		
683	Wheat	1,151	+ 468
	Rice	3,238	+ 2,555
	Alfalfa	6,884	+ 6,201
	AREA 2		
1,415	Rice	5,037	+ 3,602
	Alfalfa	10,707	+ 9,292
	AREA 3		
2,271	Wheat	4,217	+ 1,946
	Alfalfa	25,240	+22,969
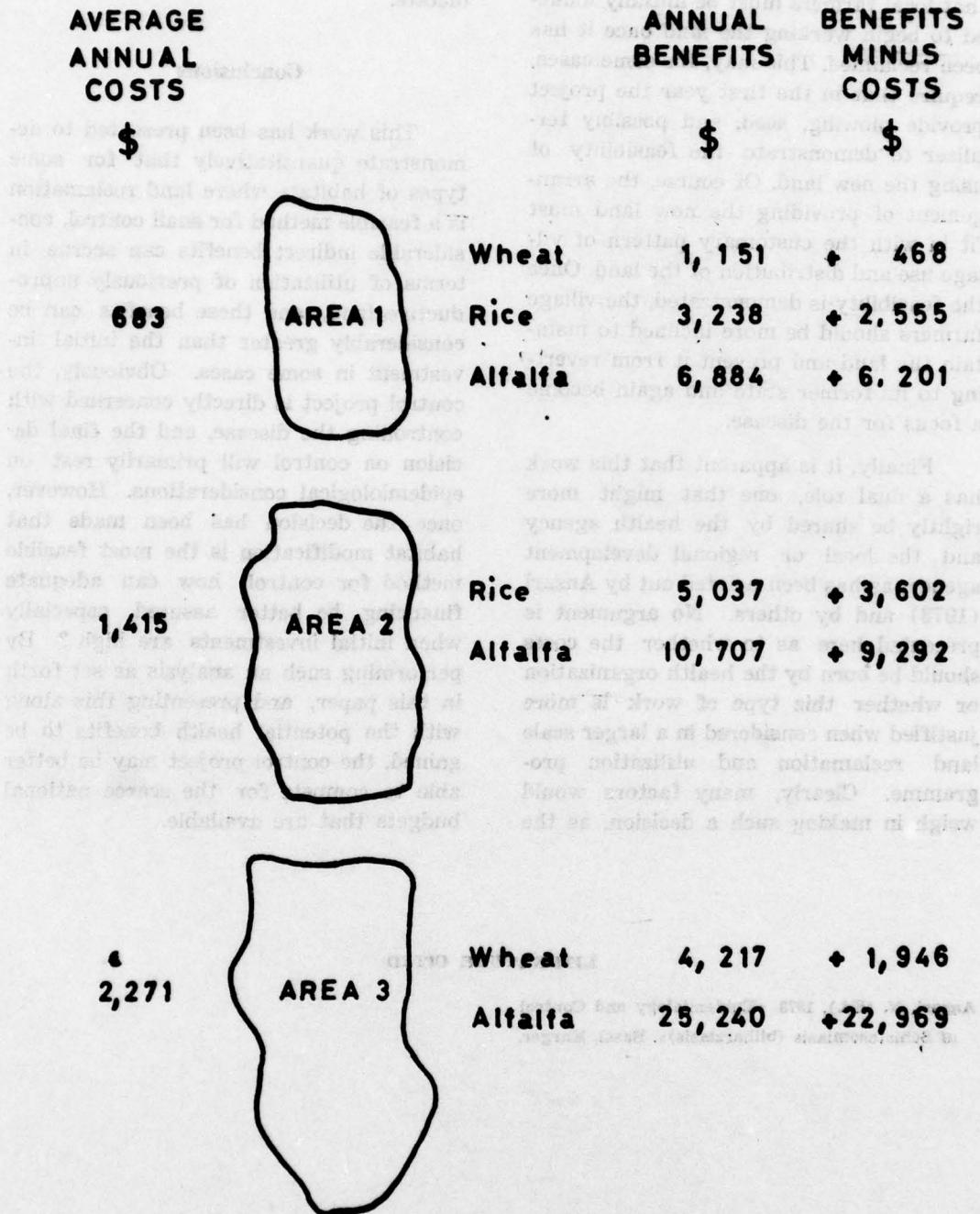
Fig. 2. Cost benefit analysis for the three reclaimed areas.

It has been found for other similar work performed in the Khuzistan area, that local farmers must be initially induced to begin working the land once it has been reclaimed. This may, for some cases, require that in the first year the project provide plowing, seed, and possibly fertilizer to demonstrate the feasibility of using the new land. Of course, the arrangement of providing the new land must fit in with the customary pattern of village use and distribution of the land. Once the feasibility is demonstrated, the village farmers should be more inclined to maintain the land and prevent it from reverting to its former state and again become a focus for the disease.

Finally, it is apparent that this work has a dual role, one that might more rightly be shared by the health agency and the local or regional development agency, as has been pointed out by Ansari (1973) and by others. No argument is presented here as to whether the costs should be born by the health organization or whether this type of work is more justified when considered in a larger scale land reclamation and utilization programme. Clearly, many factors would weigh in making such a decision, as the extent of work, budget and personnel restrictions, local development goals, etc., dictate.

Conclusions

This work has been presented to demonstrate quantitatively that for some types of habitats where land reclamation is a feasible method for snail control, considerable indirect benefits can accrue in terms of utilization of previously unproductive land, and these benefits can be considerably greater than the initial investment in some cases. Obviously, the control project is directly concerned with controlling the disease, and the final decision on control will primarily rest on epidemiological considerations. However, once the decision has been made that habitat modification is the most feasible method for control, how can adequate financing be better assured, especially when initial investments are high ? By performing such an analysis as set forth in this paper, and presenting this along with the potential health benefits to be gained, the control project may be better able to compete for the scarce national budgets that are available.

LITERATURE CITED

Ansari, N. (Ed.), 1973. «Epidemiology and Control of Schistosomiasis (bilharziasis)». Basel, Karger.

RESULTS OF A WATER SUPPLY SCHEME ON THE TRANSMISSION
OF *SCHISTOSOMA MANSONI*

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One method for reducing the transmission of *Schistosoma mansoni* aimed at preventing exposure to infection, is the provision of a convenient and reliable water supply. Such a water service in rural areas makes it unnecessary for the population to enter natural water harboring the infective larval stage of the schistosome. In St. Lucia a household water supply is being provided in five rural settlements.

Pre-control studies including geographic surveys, population census, parasitological surveys and water contact observations were made for 3 years prior to control operations. These data served as the basis for the design and construction of three separate water supply systems supplying on-premise water to each household in the five settlements. In addition five public laundry-shower units and three swimming pools have been constructed at the strategic locations.

The three water systems became operational over a period of just under 2 years beginning in June 1970. The three systems have a total design capacity for a population of 4500. Present population served is just over 2000 persons or nearly 400 households. Each household is provided with a limited flow (Fordilla) faucet, which automatically shuts off the flow after 5 to 6 litres of water have been discharged ; for reactivation the push button is released and pressed down again. This type of faucet furnishes an unlimited amount of water but cannot be left open to waste water. Plastic (PVC) pipes which were used for the water distribution line proved to be an advantage in savings on labor land material costs.

The public laundry-shower units consist of a simply hip-roof structure with either 6 or 8 concrete wash tubs mounted on a centre bench. Shower stalls are constructed of concrete block wall and partitions. The materials and equipment costs at the time of construction amounted to $ 37,300 (U.S.). Design, supervision and installation labor, which are subject to considerable variations from region to region, are not included in this figure. Annual maintenance costs amounted to about $ 2.00 per capita served prior to increased costs for energy.

On the northern side of the same valley are six other settlements with a population of 2000 served with a public stand-pipe water system consisting of only 16 standpipes. These settlements serve as the comparison area.

Pre-control annual stool surveys (between 1968 and 1970) in the water supply and comparison areas showed similar levels of incidence, intensity and prevalence of infection.

Post control water contact studies showed a 95% reduction in the number of persons observed in the river compared with the pre-control findings. This confirms that persons in this area when offered an alternate water supply along with some health education, changed their habits and accepted the more convenient supply.

Incidence of new infections among 0-10 year old children in the water supply area fell from 31% in 1970 to 14% in 1974. By 1974 this reduced incidence resulted in a drop in the intensity of infection from 31 to 21 eggs/ml* among children in the 0-14 age group and prevalence fell from 50% to 29% in the same group during this same period (Table 1).

TABLE 1. Changes in Indices of Transmission

	Water Supply Areas		Comparison Areas	
	1970	1974	1970	1974
Incidence (%) (0-10 yrs)	30.6	13.8	23.8	29.0
Prevalence (%) (0-14 yrs)	49.7	28.5	35.5	54.4
Intensity Eggs/ml feces* (0-14 yrs)	35.5	22.4	31.6	51.7

* Geometric mean.

At the same time incidence has increased in the comparison area from 24% to 29%. Prevalence and intensity of infection have risen in this area from 35% to 54% and from 32 to 52 eggs ml respectively.

Sentinel snail exposures have shown a reduction in the infection rate from 0.55% to 0.21% from 1971 to 1974 in the water supply area, while in the comparison area the rate of infection increased from 0.17% to 0.79% during the same period.

It should be noted that one of the comparison settlements, where all indices of infection have increased between 1970 and 1974, is only physically separated by a distance of approximately 1 kilometer from the first water supply settlement, and that of the five settlement for which results are available, only one has had water for the 4 years; three have had water 3 years and one for only 2 years.

The daily mean water usage in the control area is 55 litres per person; an increase of 35 litres over presupply estimates indicating improved hygiene and health practices. This is probably responsible for greater weight increments of infants in the water supply area over the infants in the comparison area.

The results of the water supply project suggest that old rural customs, such as washing and bathing at river sites, can be changed when acceptable alternatives are provided and that a reliable adequate, conveniently delivered water supply can do much to reduce transmission of schistosomiasis.

Aside from the direct health benefits associated with safe water supplies there are many other «quality of life» benefits. Thus water supply should be considered as a method of control of schistosomiasis in some areas and since it is not disease specific it may be much more acceptable to authorities who are also interested in the broad spectrum of social and health programmes.

STUDIES ON ANTAGONISM BETWEEN LARVAL SCHISTOSOMES AND ECHINOSTOMES IN THE SHARED SNAIL HOST

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During investigations of larval trematodes in freshwater snails performed by the joint Czechoslovak-Egyptian team[*] in the vicinity of Cairo, from 1971 to 1973, it was found that the occurrence of snails simultaneously infected with sporocysts, rediae, or cercariae of two different trematode species is scarce in nature. Similar observations have been made in the field by several other authors (e.g. Cort et al., 1937 ; Wesenberg-Lund, 1934 ; Porter, 1938 ; Fain, 1953 ; Nasir, 1962 ; Boray, 1964, 1967), but detailed experimental studies on this subject have been started only in the last decade by Prof. Lie and his coworkers. The ultimate aim of these studies was the possible utilization of this interspecific competition in the biological control of schistosomes pathogenic to man. Antagonistic interactions between *Schistosoma mansoni* and several strigeid or echinostome trematodes have already been proved by Lie (1967, 1969) ; Heyneman et al. (1972) ; Basch et al. (1969) and others ; similar experiments with other species of human schistosomes have not been carried out. Our studies performed near Cairo in the vicinity of the village of Warak El Arab revealed that the schistosome intermediate hosts in

Egypt, i.e. the snails *Bulinus truncatus* and *Biomphalaria alexandrina*, were often infected with echinostomes the adults of which are the parasites of various rodents and birds. These trematodes have a relatively short life-cycle, a wide range of definitive hosts and can be easily maintained in the laboratory.

Our studies of interactions between schistosomes and echinostomes during their development in the shared snail host were based on two series of experiments. The first series was established for studies on antagonism between *Schistosoma haematobium* and *Echinoparyphium recurvatum* in the snail *Bulinus truncatus*, while the second one was directed to verification of the relationship between *Schistosoma mansoni* and *Echinostoma revolutum* during their simultaneous development in *Biomphalaria alexandrina*. The results of these experiments have already been published in the journal Folia parasitologica last year (Moravec et al., 1974 ; Barus et al., 1974) and are, therefore, mentioned only briefly.

In both series of experiments, the local species of freshwater snails, *Bulinus truncatus* and *Biomphalaria alexandrina*,

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[*] The team was established according to the agreement between the Czechoslovak Academy of Sciences and the Egyptian Ministry of Scientific Research.

were used ; these are the only intermediate hosts of *Schistosoma haematobium* and *S. mansoni,* respectively, in the region. All experiments were carried out in the laboratory.

In studying the interaction between *Schistosoma haematobium* and *Echinoparyphium recurvatum,* the snails were divided into 4 experimental and 3 control groups. The experimental snails were (1) infected simultaneously with the miracidia of both trematode species, (2) and (3) superinfected with *E. recurvatum* on *S. haematobium* at intervals of 20 and 30 days and (4) superinfected with *E. recurvatum* on *S. haematobium* at the time of cercarial shedding. The control groups consisted of (1) uninfected snails and snails with a single infection with (2) *S. haematobium* or (3) *E. recurvatum*). Then the individual experimental and control groups were compared with regard to the mortality of snails, values of so-called infection rates (i.e. percentage of snails shedding cercariae out of the total number of surviving snails in the group), time span for cercarial release, etc. In the groups with double infection, schistosome cercariae appeared only in the group with a 30-day interval between the exposures to miracidia of both trematode species, but the period of cercarial production was 6 times shorter than in the control group with a single infection with *S. haematobium*. The results suggest that a short-term production of schistosome cercariae occurs only when the snail infected with echinostomes has already harboured advanced stages of schistosomes, but even then the schistosome infection is eventually suppressed by the echinostome. Our experiments revealed the capability of *E. recurvatum* to suppress completely the infection with *S. haematobium* even in the snails which are already shedding the cercariae of

schistosomes at the time of superinfection. Similar results were achieved in the second series of experiments, in which interactions between *Schistosoma mansoni* and *Echinostoma revolutum* were studied in the snail *Biomphalaria alexandrina*.

Our experiments confirmed that interspecific antagonism occurs during the simultaneous development of echinostomes and human schistosomes in the shared intermediate host ; the echinostomes are always the dominant species, suppressing effectively the schistosomes at any stage of development. The mechanism of antagonism between echinostomes and schistosomes was studied by Lie (1967), Lie et al. (1968) and others, who observed the predation of echinostome rediae, which damage mechanically and consume the sporocysts of schistosomes. Echinostome cercariae too seem to participate in this direct antagonism ; while re-entering the snail and encysting in its tissues they also damage the sporocysts of schistosomes. Indirect antagonism manifested by retarding of the larval development was not observed.

When considering the possible utilization of echinostomes for the biological control of schistosomes, it is of importance that the mere presence of these larval trematodes, which are highly pathogenic to the snails, results in a high rate of snail mortality, in their restricted growth, fertility, resistance to external influences, etc. A high mortality of snails was observed in all experimental and control groups with echinostome infection.

Our experiments confirmed antagonism between the local species of echinostomes and schistosomes, which could be utilized for the biological control of schistosomes in Egypt and probably in other countries as well. However, we are aware

of the fact that a series of field experiments at the sites with schistosome occurrence are necessary for evaluating the effectiveness of these methods. We assume that the application of echinostomes in the control of schistosomiasis would be primarily possible at the places having the character of a focus, as, for example, the locality found by us (see Rysavý et al., 1974) near the village of El Karatein. To ensure a sufficient infection of snails with echinostomes it would be necessary to supply the eggs of the trematodes from laboratory breeding or to maintain a sufficient density of definitive hosts in the respective localities. In the latter case, the introduction of duck breeds on free runs to the localities highly infested with schistosomes seems to be advantageous. The duck may become a definitive host for both *Echinoparyphium recurvatum* and *Echinostoma revolutum,* thus making possible the control of *Schistosoma haematobium* and *S. mansoni* at the same time. Moreover, ducks feed on watersnails and, hence, may considerably reduce the local snail populations. To increase the efficacy, this procedure might be applied in combination with other measures.

LITERATURE CITED

Baruš, V., Moravec, F., Rysavý, B. & Yousif, F., 1974. Antagonism of Echinostoma revolutum against Schistosoma mansoni in the snail Biomphalaria alexandrina. Folia parasitol. (Praha) 21: 143-154.

Basch, P.F., Lie, K.J. & Heyneman, D., 1969. Antagonistic interaction between strigeid and schistosome sporocysts within a snail host. J. Parasit. 55: 753-758.

Boray, J.C., 1964. Studies on the ecology of Lymnaea tomentosa, the intermediate host of Fasciola hepatica. I. History, geographical distribution, and environment. Australian J. Zool. 12: 217-230.

Boray, J.C., 1967. Host-parasite relationship between lymnaeid snails and Fasciola hepatica. Veterinary Medical Reviews, «The reaction of the host to parasitism», pp. 132-140.

Cort, W.W., McMullen, D.B. & Brackett, S., 1937. Ecological studies on the cercariae in Stagnicola emarginata angulata (Sowerby) in the Douglas Lake region, Michigan. J. Parasit. 23: 504-532.

Fain, A., 1953. Contribution à l'étude des formes larvaires des Trématodes au Congo belge et spécialement de la larve de Schistosoma mansoni. Mém. Inst. Roy. Colon. Belge 22: 1-312.

Heyneman, D., Lim, H.K. & Jeyarasasingam, U., 1972. Antagonism of Echinostoma liei (Trematoda: Echinostomatidae) against the trematodes Paryphostomum segregatum and Schistosoma mansoni. Parasitology 65: 223-233.

Lie, K.J., 1967. Antagonism of Paryphostomum segregatum rediae to Schistosoma mansoni sporocysts in the snail Biomphalaria glabrata. J. Parasit. 53: 969-976.

Lie, K.J., 1969. Role of immature rediae in antagonism of Paryphostomum segregatum to Schistosoma mansoni and larval development in degenerated sporocysts. Z. Parasitenk. 32: 316-323.

Lie, K.J., Basch, P.F. & Heyneman, D., 1968. Direct and indirect antagonism between Paryphostomum segregatum and Echinostoma paraensei in the snail Biomphalaria glabrata. Z. Parasitenk. 31: 101-107.

Moravec, F., Baruš, V., Rysavý, B. & Yousif, F., 1974. Antagonism of Echinoparyphium recurvatum against Schistosoma haematobium in the snail Bulinus truncatus. Folia parasitol. (Praha) 21: 127-141.

Nasir, P., 1962. Further observations on the life cycle of Echinostoma nudicaudatum Nasir, 1960 (Echinostomatidae: Trematoda). Proc. helm. Soc. Wash. 29: 115-127.

Porter, A., 1938. The larval Trematoda found in certain South African Mollusca with special reference to schistosomiasis (bilharziasis). Publ. S. Afr. Inst. med. Res. 42 (8): 1-492.

Rysavý, B., Baruš, V., Moravec, F. & Yousif, F., 1974. On some problems of the biological control of human schistosomes in Egypt. Folia parasitol. (Praha) 21: 161-168.

Wesenberg-Lund, C., 1934. Contributions to the development of the trematoda Digenea. Part II. The biology of freshwater cercariae in Danish freshwaters. Mem. Acad. Roy. Sci. Lett. Danemark, Copenhagen, Sec. Sci., Ser. 9, 5: 1-223 + 39 pls.

INDUCTION AND MODIFICATION OF IMMUNITY IN THE SNAIL HOST
OF *SCHISTOSOMA MANSONI* AND ITS POSSIBLE EFFECT
ON THE BIOLOGICAL CONTROL OF THIS PARASITE

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ABSTRACT

Using the echinostome trematode, **Echinostoma lindoense**, and the snail host, **Biomphalaria glabrata** (New World host of **Schistosoma mansoni**), we have undertaken over the past year a series of experimental infections initiated by one of us (L.K.J.) that raise new questions and challenge some concepts on invertebrate immunity. We have been employing the above infection system to investigate trematode-mollusk interactions, trematode-trematode competition and antagonism, specific mollusk immunity, and the feasibility and limitations of non-schistosome trematode infections for the biological control of schistosomiasis. The following observations will be reviewed and their significance discussed in terms of these phenomena:

1. Cannibalism among successive echinostome infections;

2. Presence of an amebocyte-producing organ responsive to specific infection challenge;

3. A specifically induced amebocyte mass that destroys the sporocyst stage of an echinostome parasite in the snail heart;

4. Induction of specific tissue sensitization in homologously challenged snail hosts;

5. Demonstration of degrees of specificity and reproducibility of this molluscan immune response;

6. Demonstration of similarities and distinctions between natural and acquired resistance in our colony of this host snail;

7. Control or midification of natural and acquired resistance.

Under the limitations of our experimental system, we have been able to demonstrate, reproducibly, a specific acquired resistance to a trematode challenge infection, with partial resistance to closely related flukes, and little or none to more distantly related ones. This induction of a specific host tissue sensitization is correlated with enlargement of an amebocyte-producing organ in the snail, intensity of the granulomatous tissue response, and encapsulation and destruction of echinostome sporocysts in the heart by an amebocyte aggregation. After destruction of the parasites in the snail heart, and after encapsulation and phagocytosis of the migrating sporocysts in the host tissues or blood vessels, the amebocyte organ recedes and the intracardiac amebocyte aggregation breaks up and disappears in the host circulation.

Presence of a demonstrable and specific immunity in a snail is of considerable intrinsic interest but also has a bearing on the potential application of heterologous trematode antagonism as a biological control mechanism. The approach is under exploration as a possible adjunct method for regionally localized control of schistosomes within the host snail.

BIOLOGICAL CONTROL OF SCHISTOSOMIASIS BY GENETIC
MANIPULATION OF INTERMEDIATE-HOST SNAIL POPULATIONS

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ABSTRACT

The possibility of disrupting the life cycle of schistosomes by reducing the rate of their transmission through the intermediate-host snail population is examined critically. Several lines of evidence indicate that snail populations are genetically variable for genes determining levels of susceptibility (or resistance) to infection by schistosomes. Biological control of schistosomiasis by genetic manipulation of host snail populations would involve introducing numbers of conspecific but refractory snails into the host snail population at a site of disease transmission. Snail infection rates would then decrease over a period of several years as hybridization between refractory and local snails, and competition between refractory and susceptible genotypes proceeded.

Preliminary computer simulations based on simple models of the population genetics and ecology of this evolutionary interaction have been undertaken. Results suggest that this method of biological control may be effective at sites where snail populations are relatively small and localized, snail infection rates are relatively high (above 5%), and where there is no recruitment to the schistosome population from outside the control area. Under such circumstances it may be possible to halve snail infection rates in as few as 20 generations (2-5 years, depending on species and local generation time). This suggests that schistosomes could be eradicated locally before they can evolve to counter to the changes in snail susceptibility.

These results, coupled with the possible advantages of this technique over existing methods of snail control, and the costs and difficulties associated with control measures involving social change, encourage us to examine the proposed technique in more detail. Unfortunately, much of the data required to assess the feasibility of this technique are unavailable. We have consequently begun to investigate the population genetics and ecology of selected populations of **Biomphalaria glabrata** and **Bulinus truncatus**. We are presently trying to measure the genetically controlled variability in susceptibility to infection within natural populations of snails and flukes.

Ultimately, we will employ a selection procedure and establish refractory stocks of snails, and proceed to study the ecology and behavior of mixed high- and low-susceptibility stocks in the laboratory. These studies should lead to the development of snail stocks and protocol for the critical field-testing of this method of biological control.

COMPETITIVE INTERACTION : AN ADJUNCT OR ALTERNATIVE TO CONTROL OF HOST SNAILS BY MOLLUSCICIDES

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During the past 50 years, molluscicides have been the «method of choice» for controlling the snail hosts of schistosomiasis. Although a degree of success has been accomplished in some limited areas, molluscicides have been a disappointment in large scale projects and Gilles et al. (1973) noted, «It is our opinion that in areas — e.g. the Nile Delta — where all the multicausal factors known to promote the disease are present, it is very unlikely that one single method of control (molluscicides) will be capable of controlling schistosomiasis». Moreover, present trends suggest that the chemical industry will sharply limit, or, perhaps, cease the development of new compounds. In this age of environmental awareness, there is an ever pressing demand for alternatives to toxic chemicals. One possible alternative and one which has received little attention is biological control. As noted by Van den Bosch & Messenger (1973), «Biological control is a natural ecological phenomenon which, when applied successfully to a pest control problem, can provide a relatively permanent, harmonious, and economical solution». As such, the method appears highly applicable for the control of schistosome-bearing snails.

Previous efforts in the biological control of schistosome-bearing snails have concentrated, in the main, on attempts to discover suitable predators and parasites. Unfortunately, other approaches have received little attention. Competitive exclusion has been studied intensely by entomologists and ecologists, but has received little serious consideration with respect to molluscs.

Although studies on competitive exclusion in host snails are almost non-existent, there is some circumstantial evidence which suggests that the principal may be operational in nature. The absence of **Biomphalaria glabrata**, the major snail-host of S. mansoni in the Neotropics, from the Greater Antilles and Central America where species of Helisoma are the dominant planorbid, may represent an example of exclusion. Moreover, in Puerto Rico where **Helisoma** and **B. glabrata** both occur, they rarely inhabit the same body of water. Recent studies by Egyptian workers (Ayad et al., 1970 ; Abdallah & Nasr, 1973) appear to confirm the fact that **Helisoma** may inhibit the growth of schistosome-bearing snails in the laboratory. It has been suggested by Wright (1973) that competitive exclusion should be considered as one factor which may account for the distribution of the three schistosome-host species in Brazil.

Recent studies in our laboratory have shown that certain strains and species of **Helisoma** compete effectively with **B. glabrata** populations and markedly reduce or eliminate the schistosome snail-host. The process becomes more effective when the selective force of infection is introduced into the system. In addition, we have evidence which suggests that **B. straminea** competes with **B. glabrata** and will, in time, replace populations of the latter. One factor which appears to contribute to the competitive effectiveness of **B. straminea** is its ability to initiate the «crowding effect» upon population of **B. glabrata** ; thus, reducing its intrinsic growth rate and level of fecundity. In one series of experiments, it was observed that the **B. glabrata** population was completely eliminated after 6 months of competitive interaction.

LITERATURE CITED

Abdallah, A. & Nasr, T., 1973. Helisoma duryi as a means of biological control of schistosomiasis vector snails. J. Egypt. med. Assoc., 56: 514-520.

Ayad, N., Mousa, A.H., Ishak, M.M., Yousif, F. & Zaghloul, S., 1970. A preliminary study on biological control of the snail intermediate host of schistosomiasis in U.A.R. by Helisoma duryi snails. Hydrobiologia, 35: 196-202.

Gilles, H.M., Zaki, A.A., Soussa, M.H., Samaan, S.A., Soliman, S.S., Hassan, A. & Barbosa, F., 1973. Results of a seven year snail control project on the endemicity of Schistosoma haematobium in Egypt. Ann. trop. Med. Parasit., 67: 45-65.

Van den Bosch, R. & Messenger, P.S., 1973. «Biological Control». Intertext Ed. Publ., New York & London, 180 pp.

Wright, W.H., 1973. Geographical distribution of schistosomes and their intermediate hosts. Chapt. 3 in: «Epidemiology and Control of Schistosomiasis», Karger, Basel and University Park Press, Baltimore, pp. 32-249.

SUPPLY OF SAFE WATER FOR DRINKING AND OTHER DOMESTIC PURPOSES*

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ABSTRACT

Since the cercaria is the infective stage in the schistosomal cycle, our attention was directed towards finding cercaricidal agents that would have the cheapest possible cost.

Out of the various compounds screened, it was found that citric acid in a dilution of 1/4000 or better still lemon juice in the same dilution was lethal to newly shed cercariae within 20 min., although most of the cercariae dropped to the bottom of the test tube after the first 10 min. Cercariae were not affected by other acids including fuming nitric acid in 1/4000 dilution.

According to these findings we are now constructing miniature swimming pools for both children and adults and for washing and drinking, in areas that are not yet provided with a potable water supply, with water treated with lemon juice in these high dilutions. These miniature swimming pools will almost certainly distract the children from the canals.

Lemon and other citrus fruit are present throughout the countryside and therefore we would obviate the problem of introducing, distributing and controlling the dose of other imported cercaricidal agents.

We are currently screening various other cercaricidal compounds to find the most practical and safe compounds with the objective of lowering the incidence of schistosomiasis.

TITLES OF PAPERS ON OTHER TOPICS,
PRESENTED BUT NOT READ

1. METABOLIC STUDIES IN EXPERIMENTAL SCHISTOSOMIASIS

1. METABOLIC CHANGES IN EXPERIMENTAL SCHISTOSOMIASIS

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2. METABOLIC EFFECTS OF NIRIDAZOLE

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3. METABOLIC EFFECTS OF SOME ANTIBILHARZIAL DRUGS. STUDIES ON THE INHIBITORY ACTION OF BILHARCID ON SUCCINATE OXIDATION IN NORMAL RAT LIVER

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**4. STUDIES ON THE EFFECT OF NIRIDAZOLE ON THE LIPID
PATTERN IN THE PARASITE AND HOST TISSUES
IN EXPERIMENTAL *S. MANSONI***

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RECOMMENDATIONS

RECOMMENDATIONS
EPIDEMIOLOGICAL ASPECTS
OF SCHISTOSOMIASIS

It is recommended that :

1. More clinical surveys be carried out together with egg output studies to correlate intensity of infection with resulting disease in clinical and pathological terms.

2. The relative importance of schistosomiasis to other health problems be determined in endemic areas and that measurements of the impact of the problem on the community should be established as a basis for estimating its economic implications.

3. Studies be carried out on the relationship between contamination and the degree of infectivity of different transmission foci.

4. Studies should be attempted on the relationship between different patterns of cercarial exposure and the resulting intensity of human infections.

5. Well-designed epidemiological studies of prevalence, incidence and intensity of infection be made in endemic areas in an attempt to determine the mechanism of acquired resistance to schistosomiasis which may play a significant role in the epidemiology of infections and the planning and evaluation of control strategies.

6. Control measures employing available tools should be identified clearly, which will result in cost effective control and achieve predictable goals over short, medium and long-term periods.

7. The composition of any control programme should be firmly based upon local epidemiological conditions, the goal of the control effort, available resources and a feasible strategy.

8. Human behaviour should receive appropriate attention in any long-term measures for control of transmission.

9. Training programmes be developed in new pilot control schemes and existing control programmes as a matter of urgency, in order to ensure that adequate manpower resources with the necessary managerial skills are available for the expansion of control efforts.

10. Whenever feasible and comparable with governments' policy to improve the quality of life on socio-medical grounds, control measures utilizing appropriate available tools should be applied now, even though the economic implications of the problem and the cost-benefits of control may not have been established.

NOTES AND RECOMMENDATIONS
SOCIO-ECONOMIC ASPECTS
OF SCHISTOSOMIASIS

The extent to which schistosomiasis constitutes a significant public health, social or economic problem is just as variable as the extent to which conditions of transmission, intensities of infection and prevalence of overt clinical disease varies from community to community and from nation to nation. Since severity of schistosomiasis varies greatly, the extent to which programs for its control can be justified also varies, but wherever it occurs schistosomiasis is a preventable human infection which reduces the quality of human life by one degree or another.

Justification for projects which directly or indirectly can achieve control of schistosomiasis also may vary depending upon national goals and the views of individuals who participate in the decision making process. The role of the economist in decision making is to examine and to present to the highest appropriate authorities an analysis of the economic merits and demerits of expenditures in support of programmes which have been proposed by technical sectors such and agriculture, health and public works.

Assessment of projects involves not only the analysis of estimated costs and anticipated benefits but also a judgment of the extent to which expenditure for a given project will contribute toward accomplishment of national goals as compared with expenditures for other competing projects. Variation in national goals and variation in the current severity of the schistosomiasis problem in different nations, require that the justification of national schistosomiasis control efforts be considered on a case by case basis.

If public officials are to discharge their responsibilities for allocating limited budgets effectively, and if some of those limited funds are to be devoted to control of schistosomiasis, those officials will need more information than is now available about the costs of schistosomiasis control and about the multiple forms and magnitudes of benefits.

We recommend, therefore, the following :

1. The WHO should convene an expert group for the purpose of deciding on the specific forms of socio-economic effects that should be studied and on operational measurements for each. This group should meet periodically to reconsider its recommendations in

the light of actual experience with the collection of data.

2. In order to determine the relationship of different levels of schistosomal infections and disease with socio-economic effects, it would be desirable to undertake a «worst-case» empirical study. This would involve research in an area in which there is professional agreement that the intensities of infection and levels of disease are high enough for at least a significant portion of the population to permit estimates of the maximum detrimental impacts of schistosomiasis in communities which represent the upper ranges of severity.

3. A number of studies, perhaps on a random sample basis, are needed to provide estimates for a number of geographic areas on the population frequency distribution of levels of intensity of infection and clinical gradients of disease.

In summary studies are needed of (1) socio-economic effects and of (2) frequency distribution of infection intensities and clinical disease gradients. Studies of socio-economic effects would doubtless show that at sufficiently high levels of infection severity, these effects become large, but without the related frequency — distribution data, planners would not know how many people are in the large-effect class. Similarly, if only the frequency — distribution data were available, planners would not have any information as to the magnitudes of socio-economic impacts resulting from the reported infection intensities.

The needed research should require neither vast sums of money nor long delays. Until it is done, however, the question will remain, not, whether expenditures on control of schistosomiasis are

desirable, but where they contribute more to human welfare than would the other social and economic programmes that are clamoring for public funding.

RECOMMENDATIONS
MOLLUSCICIDE CONTROL OF VECTOR SNAILS AND CONTROL PROJECTS

After reviewing the situation with regard to snail control with molluscicides and noting that mollusciciding was one of the effective measures for controlling schistosomiasis, agreement was reached on the following recommendations :

1. The use of molluscicides should continue in conjunction with other recognized measures.

2. The effective use of molluscicides should be based on appropriate prior studies on snail biology, water management, irrigation practices and weed clearance.

3. Evaluation of the toxicity of molluscicides and of pathogenicity in man, domestic animals, crops and wild life should continue. Further studies on the effects of molluscicides on biota should be directed to long-term assessment of any cumulative effects. The possible development of snail resistance to molluscicides should also be investigated.

4. Focal transmission control could be used wherever it does reduce transmission and saves the costs of generalized mollusciciding.

5. Research on novel molluscicides and on new formulations of available molluscicides should be encouraged, with special attention to slow release formulations and regard to their effectiveness and toxicity.

6. In view of the increasing cost of available synthetic molluscicides,

alternative means of controlling snails by locally producible natural product molluscicides should be encouraged. Although the potencies of some plant molluscicides may not be competitive with some of the synthetic molluscicides, their use in the control of schistosomiasis in rural areas on a self help basis is recommended. Endod (*Phytolacca dodecandra*), the plant which has been most extensively investigated, offers good possibilities in schistosomiasis control and further studies on it are recommended.

7. Further attention should be paid to cost-benefit effectiveness of mollusciciding programmes.

8. On account of the seriousness of schistosomiasis as a public health problem affecting a considerable part of the world population in many countries, it is recommended that an adequate specific international fund and a programme for combatting schistosomiasis be created to assist countries where the disease is prevalent.

NOTES AND RECOMMENDATIONS
CHEMOTHERAPY OF HUMAN SCHISTOSOMIASIS

1. Noting the valuable role of specific chemotherapy in the treatment of infected individuals and for the control of infection, the group recommends the wider use of schistosomicidal drugs in combination with other control measures in well designed programmes. In view of the variations in the epidemiology of Schistosomiasis in different ecological areas, the choice of drugs, dosage and the selection of subjects should be determined in terms of the local situation.

2. Noting that the currently available schistosomicidal drugs are not ideal with regard to efficacy, ease of administration and safety, the group recommends more intensive search for new anti-schistosomal drugs.

In particular the group recommends :

a) Closer collaboration between the pharmaceutical companies and international health agencies in the development and testing of new agents, and

b) More basic science research into the biology of parasites and the mode of action of schistosomicidal drugs.

3. a) The group noted the reports of the successful use of hycanthone in the treatment of patients and in large-scale chemotherapy. In well supervised programmes the drug was effective and reasonably safe. Acute toxicity occurred in a few cases ; it would appear that the risk of this complication can be reduced by careful screening of patients and the exclusion of those who have contra-indications. There was much concern about the reports of laboratory tests for mutagenicity and there was some uncertainty about the validity of the test system in the context of human risk.

The group recommends that further studies should be carried out on this and other schistosomicidal drugs in order to obtain more meaningful assessment of the risks posed by the use of these drugs. Meanwhile, no results published to date on possible genetic damage should prohibit the continued use of hycanthone or any of the other drugs in current use .The relevant

bodies (WHO, toxicologists, geneticists, expert groups, etc.) should keep the matter under careful review collating and interpreting all relevant data for the guidance of national health authorities and physicians.

b) The group also noted the report of carcinogenic effects of niridazole in mice and recommends that further studies be done including other mammalian species. Since the drug has been widely used during the past 10 years, treated patients and populations should be monitored whenever possible to detect any possible excess of neoplasia.

c) The group strongly recommends that other schistosomicidal drugs including new agents, should be rigorously tested for mutagenic and carcinogenic effects.

d) To determine what schistosomicidal drugs can be used in a particular nation will be the responsibility of each national drug licencing authority. Great care should be exercised in the selection of drugs for large-scale chemotherapy after weighing the potential risks and benefits in the light of the best available evidence.

4. The group noted the urgent need for clinical pharmacologists with expertise in parasitic diseases who can correctly design, conduct and analyse drug trials in different populations so as to obtain accurate estimates of the therapeutic properties and side effects of parasiticidal drugs. The group recommends that initial trials should be conducted in specialist units or university hospitals under adequate monitoring safeguards ; further trials should be

conducted by experienced personnel with supervision by pear groups to ensure that high ethical standards are maintained.

NOTES AND RECOMMENDATIONS IMMUNOLOGICAL ASPECTS OF SCHISTOSOMIASIS

Immunological research in schistosomiasis has made important progress during recent years. The committee feels strongly that further concerted programmes in this field are urgently needed.

Justifications

1. Immunology of schistosomiasis opens the way for the potential immunological control for both disease and infection.

   Animal experiments proved the feasibility of this approach. Furthermore some endemic areas are unique sites for conducting studies on acquired resistance in human schistosomiasis.

2. Serology and immunodiagnosis of schistosomiasis provide opportunities for better definition of patient populations, their immunological responses, proper follow-up and their responses to therapy.

3. Gaps in our knowledge of the immunopathology of schistosomiasis prevent proper understanding of the disease process and its management. Further work is urgently needed to elucidate specific mechanisms of disease.

4. The wealth of knowledge in immunology and the clinical material available in some endemic areas can provide answers for some of the basic immunologic questions pertinent to schistosomiasis.

   The committee feels that these justifications necessitate an interdisciplinary approach, in which workers in several different disciplines of medicine and other sciences would cooperate, is needed for the success of this programme. This can be achieved by the organization and centralization of planning and of reviewing research in the immunology of schistosomiasis, and with proper follow-up procedures.

Specific Recommendations

1. *Central training and research facility*

   There is an urgent need to bridge the gap in immunological knowledge and to further research in this field. This can be achieved by the establishment of a central facility. Such a facility will be concerned with :

   a) Education and training of medical and technical persons.

   b) Research in areas related to the field of immunology of human schistosomiasis as outlined below.

   c) Acting as a reference center for supply of standardized reagents and antigens.

2. *Clinical immunology*

   Some environment areas provide a wealth of clinical material and exhibit unique aspects of schistosomiasis which necessitate thorough exploration. Among these aspects are :

   a) Does acquired resistance to reinfection develop in man ?

   A definitive study is still lacking to answer the above question. Carefully done longitudinal studies employing advanced methodology in immunology, epidemiology, transmission and water contact are needed.

   b) Furthermore, the effect of chemotherapy on acquired resistance should be evaluated.

c) Specific aspects of immunopathology of human schistosomiasis need further exploration ; of particular importance are :

   i. Liver and kidney disease and their association with the species of human schistosomes which are endemic.

   ii. The pathogenesis of the severe forms of human schistosomiasis including colonic polyposis which is prevalent in certain endemic areas.

d) More fundamental research, on an international collaborative basis, can be targeted towards basic studies on the mechanisms of immunity in human schistosomiasis and the possibility of developing protective vaccines.

NOTES AND RECOMMENDATIONS ECOLOGICAL AND HABITAT CONTROL OF SCHISTOSOMIASIS

Methods were discussed, other than chemical mollusciciding, for an integrated approach to pest management.

Because of the emphasis placed on molluscicides as a control method in the past 10-15 years, other possible methods of control have received little encouragement although they have shown potential value. The urgency for control of schistosomiasis will require in many instances that all available methods be used in an integrated programme. Such ecological methods for habitat management have been considered by the Sub-committee and it is recommended that schemes be set up to demonstrate the efficiency of control by environmental and habitat management methods (cf. Appendix).

It is appreciated that in many areas no single method of control available to-day, in particular mollusciciding or chemotherapy, will be completely successful in establishing control, and that environmental methods are no exception. Thus, additional methods such as chemotherapy and snail control must be considered an integral part of environmental methods of control.

With the object of demonstrating the role of environmental control methods, which in certain areas have been shown to be effective, it is recommended that

1. The following principles be investigated in appropriate localities :

   a) Reduction or prevention of human contact with infected water and prevention of contamination of surface waters through the provision of adequate, safe, reliable and conveniently placed water supplies and the construction of physical barriers.

   b) Reduction of snail populations by methods such as channelization of rivers and streams, weed control, as well as by other physical methods at or near transmission foci.

2. A number of items requiring further investigation, and identified so that their possible future role in control can be established, be given attention. These include sanitation, biological methods, use of natural plant products, improved design of reservoirs and irrigation systems, competition among snails, transmission dynamics, and the effect of high water temperature on snails.

3. It is also recommended that training in this field be supported and that a system for more rapid exchange of information be established.

CONTROL STRATEGY : A GENERAL STATEMENT

The development of a global control strategy or even effective control on a more restricted basis is a formidable and expensive task. It will call for long-term planning and decisions on overall strategy which include establishing criteria for selecting target areas and consideration of the applicability of currently available control methods to them ; the adequacy of financial and man-power resources and, probably most important, the identification of interest and motivation by national governments (Hoffman, 1975)*. There must be clear realisation on the part of those undertaking control measures that a long-term time-scale of sustained effort and, therefore, recurrent expenditure will be involved. It is also essential that control measures are clearly identified which will result in effective control and achieve predictable goals over short, medium and long-term periods. Snail control and chemotherapy are available to us now, but the development of habitat control by environmental changes and engineering means may involve medium or long-term planning and execution, as will the acceptance and use of alternative water supplies and environmental sanitation coupled with health education, in many places. Those who consider that raising the standard of rural health services is a panacaea for achieving control of this problem and related ones should remember that schistosomiasis transmission is characterised by its variability and complexity and re-

quires specific control inputs to achieve particular results. Nevertheless available tools cannot be effectively used unless a minimum basic health infrastructure exists in the operational area, and probably one of the greatest constraints to the progress of control in many situations will be the lack of capability to accept and apply advanced technology in the short-term even when adequate resources are available.

The adequacy of manpower resources, particularly with managerial skills, poses a serious problem and training programmes must be developed in new pilot schemes and existing control programmes. It is essential that basic research is also continued and adequately supported to assess the impact of the problem ; to improve the cost-effectiveness of available control tools and delivery systems and establish new ones ; to discover new drugs, and in this area industry has a key role to play ; and to conduct long-term studies towards developing a vaccine.

The provision of adequate financial resources to carry out the initial phases of control on a large scale will call for funding far beyond that which many individual governments can provide and aid from bilateral and multilateral donors must be properly integrated with national health budgets in order to initiate new control programmes and expand existing

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* Hoffman, D.B. (1975). Schistosomiasis Research, The Strategic Plan. The Edna McConnel Chark Foundation N.Y.

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ones where appropriate. It is vitally important that every effort is made to reduce the costs of control measures and that these are eventually transferred to national government responsibility for long-term maintenance purposes.

International agencies involved in water resources developments are now generally aware of the dangers of exacerbating levels of transmission and the need to consider the problem at the planning stage. The cost of current control programmes varies considerable but it is generally higher than many poor countries with widespread endemic schistosomiasis can afford in terms of generalised transmission control schemes. Schistosomiasis control is however believed to be within the capability of some countries that do not have programmes and it is considered that a high degree of effective schistosomiasis control is financially feasible in all commercial irrigation schemes. (WHO, 1973)*.

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* World Health Organization   (1973),   WHO Techn. Rep. Ser. No. 515.

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APPENDIX

UNITED NATIONS ENVIRONMENT PROGRAMME

ACTION PLAN FOR ECOLOGICAL AND HABITAT

MANAGEMENT OF SCHISTOSOMIASIS

Prepared

by

**Sub-committee on Ecological and Habitat Management,
International Conference on Schistosomiasis**

Cairo, October 1975

CONTENTS

I. Introduction

This report is a recommendation for the control of the parasitic disease schistosomiasis by ecological methods. Ecological methods are needed because chemical methods have become expensive and possibly damaging to the environment. The urgency for control of this disease will require in many instances that all available methods be used in an integrated programme. Emphasis is here given to ecological methods to overcome the current dependence on chemicals.

The Disease

Schistosomiasis or bilharziasis is one of the most important public health problems for many countries in the tropics. The disease affects about 200 million people in 71 countries in Africa, the Middle East, South America, the Caribbean, and in the Orient. It is a chronic insidious disease and its symptoms may be masked by other concomitant disease conditions. The disease is important with regard to debility and also because of complications which may cause permanent disability if not death. *Schistosoma haematobium* occurs in Africa and the Middle East and can cause severe irreversible disease of the urinary tract. *S. mansoni,* in Africa, South America and the Caribbean, affects the large intestines and can cause liver fibrosis with splenomegaly, the development of varicose veins in the intestinal tract, and in the terminal phases, ascites *S. japonicum,* occurs in parts of the Orient. Like *S. mansoni,* it can cause hepatosplenic disease, and is generally considered more severe than *S. mansoni.* In addition it affects the central nervous system more frequently than the two other schistosome infections.

Control of the Disease

It is generally accepted that no single method will be completely successful in controlling schistosomiasis transmission and environmental or ecological methods are no exception to this. The role of chemotherapy alone in control has not been fully evaluated, but snail control programmes are now being supplemented in many areas with newly developed chemotherapeutic agents, and they should be considered as a component of environmental control schemes. In addition to reducing the number of schistosome eggs contaminating the environment by the improvement of sanitation, treatment of infected persons has the distinct immediate impact of reducing disease and possibly the occurrence of disabling complications.

Purpose of this Report

This report was begun as the result of deliberations by Consultants of the United Nations Environment Programme. It was finalised at the International Conference on Schistosomiasis, held in Cairo in October 1975 as an action plan for a transnational control of schistosomiasis.

II. Proposed demonstration projects for Schistosomiasis control using environmental and ecological methods

The control of schistosomiasis is an urgent requirement for improving health in many tropical countries. The Sub-Committee on Ecological and Habitat Management proposed environmental methods which may be employed in projects for the control of schistosomiasis. This exercise was initiated when the Governing Council of the UNEP specifically requested the Executive Director to investigate methods, other than chemical, for an integrated approach to pest management. It is unfortunate that in the past, the use of chemicals for snail control has been over-emphasized in several large-scale programmes and it is hoped that careful

examination of the relative advantages of environmental methods will lead to more integrated programmes for schistosomiasis control. It is stressed that community health education must be an integral part of any scheme to control schistosomiasis with these methods.

Environmental methods of schistosome control must not be considered in isolation from the people who interact with them. Water supply and sanitary facilities need not only be built but also maintained and used. This involves changing human behaviour and too little is known about how to do this effectively and in a long-lasting manner. All research projects and operational programmes on environmental schistosome control must include adequate and rigorous study of relevant aspects of human behaviour change.

The following principles were considered and recommended as a basis for designing a programme of demonstration projects for control :

1. reduction or prevention of contact with cercarial-infested water by the provision of

   a) safe water supply

   b) physical barriers

2. reduction of snail populations by

   a) physical methods

   b) weed control

   c) channelization of rivers and streams

3. reduction of contamination of surface water.

Most of these methods are of proven value and have already been successfully employed in limited areas.

The proposed projects must be long-term undertakings since schistosomiasis

control is accomplished by patient, careful work, not by short spectacular sorties. Programmes should include appropriate periods of basic epidemiological data, planning, intervention and post-intervention observations. The costs and benefits of each project should be measured in a comparable way to provide data for future planning by calculating benefits. It should be noted that some of the proposed environmental methods have multiple benefits in terms of human health or food production and less potential for harmful side-effects than do other methods.

For the implementation of the programme of demonstration projects, the involvement of the local community as well as the use of local materials and expertise should be vigorously encouraged.

A control programme for transnational application must necessarily embody a number of measures which may be implemented singly or in combination and adapted to specific situations as exist in the field. And while the demonstration projects outlined may emphasize single forms of approach to control, it must be stressed that they should be implemented with other methods which are applicable and practical in the area. Although there is hardly any substitute for competent ecological evaluation of each local schistosomiasis problem in order to decide on the most effective control mechanism with least cost and damage to the environment, it must be emphasized that no one single method is effective in schistosomiasis control.

Specific locations where such projects may be developed, based on appraisals by representatives from each country are listed at the end of each section. These locations must be reviewed in detail prior to implementation.

1. Proposed demonstration project 1 ; To effect prevention of human water contact by the provision of an adequate water supply

The following guidelines are recommended for water supplies designed to prevent or reduce human contact with natural waters infested with cercariae.

Source of water

Water may be obtained from streams or rivers, storage or surface reservoirs, surface water intakes or dams, wells, and irrigation canals. Rainfall catchments may be feasible in areas with appropriate weather patterns and roof catchments may provide another source. Imaginative approaches both to design of facilities and funding should be encouraged.

Protection of water source

Whenever possible, human contact with the source of water should be prevented. Catchment areas and reservoirs should be protected from contamination. Fencing surface waters may be necessary and where wells are used they should be protected from surface pollution. Intakes from impoundments should be located away from the shore and in the deeper sections of the reservoir.

Quantity

An abundant supply of reasonably clean water is more important than high quality water in limited quantities. Systems that are carefully designed to eliminate water wastages, using limited flow faucets or other water saving devices may operate on the basis of 60 litres per capita per day. Where ordinary faucets are used, the design may need to provide 180-200 litres per capita daily since such faucets are frequently left to run especially when water is provided without charge.

Treatment of the water required will depend on the source of the supply. Water from properly protected wells should require no treatment. In other instances, filtration galleries or sand filters may be needed. Sedimentation chambers may be required where high silt content is a problem.

Storage

Storage of domestic water supplies should be provided whenever necessary to ensure an adequate and reliable supply. In addition to main reservoir storage, there is a need for in-line storage tanks. Capacities that provide a 1-2 day supply should be adequate for in-line storage. Retention of water for 2 days would ensure that cercariae would perish.

Distribution system

Delivery of the water should be to a convenient point with special attention given to ensure that the location is *more convenient* than the infested river or canal. Delivery should be to each household whenever possible since hand carried water is frequently contaminated and never available in quantities adequate for good personal hygiene. Whenever this is not possible there should be no more than 10 households for each delivery point. When wells are used, the water should either be piped to convenient points or the number of wells increased to provide convenient distribution. The elimination of water wastage at the hydrant, whether it be a public or a private outlet, is very important. Water left to run may create swampy areas which are suitable snail habitats and mosquito breeding sites. Therefore, these hydrants should be located on solid, well drained sites with some hard surfacing of the area immediately surrounding the hydrant. The use of the limited flow type of faucet is especially suited for these

installations. This faucet provides all the water needed, but cannot be left open to run unattended. In all cases, adequate provision for disposal of waste water is essential.

Laundry and Bathing Facilities

For schistosomiasis control, rural community water supply systems must include clothes washing and bathing facilities. These can be laundry-shower units of simple design with 6-8 tubs, preferably under a simple roof, and 4-6 shower stalls. These units should also be more conveniently located than the canal or river. For rural villages an appropriate ratio of one wash tub for each 10 households seems to be an adequate design.

Maintenance and Education

Maintenance must be considered equally a part of the water system as items such as pipes, faucets and pumps. No system will remain operational without regular routine maintenance. Full acceptance of facilities by the community is essential and these assets should be designed and built according to the habits and preferences of the people who are going to use them. Education on the proper utilization of the water supply for maximum health benefits should be part of the project and this could make maintenance easier. To help in education and to set an example, water should be provided for schools, and children instructed in its use for personal hygiene.

Cost of construction and maintenance

The cost of construction will vary between U.S. $ 3 to $ 10 per capita and should be minimised by use of local materials and community labour. Each

system must be built to suit the local situation, utilizing imaginative design. There should be a permanent governmental agency responsible for these systems. It should be made aware that the systems are for *disease control* and not simply for the convenience of the population. Community efforts should be incorporated as much as possible to provide an inexpensive permanent system of maintenance.

2. Proposed demonstration project 2 ; To effect prevention of human water contact by the provision of physical barriers

A supplementary method for prevention of human contact with water is the provision of physical barriers to prevent or make it more difficult for the human population to enter or use unsafe water supplies.

Barriers which have been used with documented success include fences of heavy brush, covered or piped channels, and other deterrents to human entry to surface water such as bridges, piers, bulkheads and similar structures to eliminate water contact. The sitting of human settlements, schools, and other population centres at least 500 meters from shores or channels creates a «distance» barrier thereby discouraging indiscriminate use and contamination, especially by children.

Because of the expenses for some of these measures, they might be necessity be limited to «focal» application in transmission zones. In addition to preventing cercarial contact, these measures also tend to reduce excretal contamination of surface water.

MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A
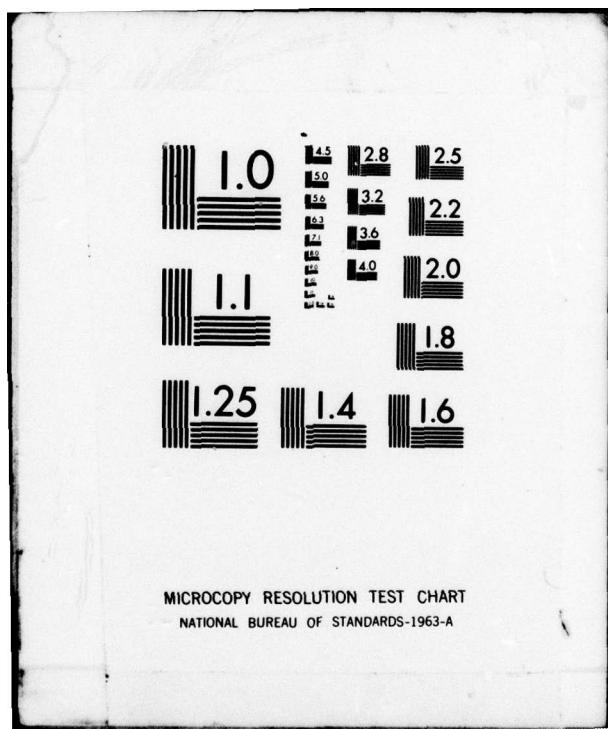
TABLE 1. Demonstration Project 1: Proposed Locations.

Country	Location	Type of project	Local contact
Nigeria	Sokoto	Piped water and bore holes latrines	Permanent Secretary, Federal Ministry of Health, Lagos Attention: Chief Health Officer cc. D. A. Lucas Dr. E.A. Smith Dr. F. Ukoli
Sudan	Gezira	Add water supply to present area of control at several villages	Dr. A.H.S. Omer, Director, Institute of Tropical Medicine P.O. Box 1304 Khartoum
	Western Sudan	Replace small natural pools with protected water supply	Dr. A.H.S. Omer and Dr. Abdel Rahman El-Tom
Brazil	Alagoas and Pernambuco	Water supply in the valley of the Mundau River, with F.S.E.S.P. and S.U.C.A.M.	Dr. Ernani Mota Superintendencia de Campanhas de Saude Publica Ministerio de Saude Esplanada dos Ministerios Blocco 11, Brasilia
Uganda	Panyagoro, Pakwach on Albert Nile	Provide water to villages near Nile as alternate supply	Dr. V.L. Ongom, Head Institute of Public Health, Makerere University P.O. Box 7072, Kampala
Liberia	Upper Lofa County	Rural water supply	Minister of Health and Liberian Institute of Bio-medical Research c/o Ministry of Health, Monrovia
Philippines	Mindoro Oriental Development Project	Add water supply to present programme of snail control and chemotherapy	Dr. A.T. Santos Jr. Executive Director, National Schistosomiasis Control Commission, Manila

TABLE 2. Demonstration Project 2: Proposed Location.

Country	Location	Type of Project	Local Contact
Nigeria	Urban Ibadan	Channelization of streams and construction of pedestrian bridges	Dr. E.A. Smith, Federal Ministry of Health, Lagos.

3. Proposed demonstration project 3; Physical measures for the reduction of snail populations, snail habitat destruction in irrigation schemes

Many water resources systems, both natural and man-made, can be constructed or altered to create habitats less favourable to snails.

Specifically for irrigation schemes, potential measures include drainage, stream channelization, paving or lining of streams and canals, land levelling and filling to eliminate low spots, seepage control, underdrains, piped or covered canals and drains, drainage of borrow pits, and improved water management.

The institution of irrigation techniques such as sprinkler and drip irrigation and covered canals which reduce the risk of creating snail habitats in irrigation schemes will contribute to schistosomiasis control. Funding agencies, international or bilateral, should require applicants to include submission of «health impact» statements as a part of irrigation and other proposed water development projects. As in the case of barriers to human contact these measures might be limited to «focal» application near human settlements with potential for schistosome transmission. The use of local materials should be investigated for these measures.

In assessing such programmes based on physical measures, careful attention should also be given to their effects on water management, agricultural productivity, land use, and disease, especially mosquito control, as well as adverse ecological impacts that they may engender. Benefits should then be attributed to the proper categories.

Programmes which modify habitats physically require comprehensive and sound background information on ecological features and possible adverse effects must be considered.

4. Proposed demonstration project 4; Physical measures, reduction of snail populations by weed control

Permanence and increase of snail populations depend on the food and shelter provided by aquatic weeds, thus the elimination of weeds from snail habitats is a method of snail control which can be used with some degree of certainty. Since weed control is also necessary in irrigation and hydroelectric power systems, it is recommended that snail control by this method be combined with programmes already underway.

TABLE 3. Demonstration Project 3: Proposed Location.

Country	Location	Types of Project	Local Contact
Brazil	Caatinga do Moura Bahia	Redesign Primitive Irrigation System	Dr. Aluizio Prata, Faculdade de Ciencias da Saude Universidade Federal de Brasilia

In reservoirs, the stranding technique for floating vegetation has been developed. This has been especially practical in hydro-electric reservoirs of moderate size in the western hemisphere. It may well be applicable elsewhere but the hazard of creating breeding sites for malaria vectors such as *Anopheles gambiae* must be avoided. Mechanical cutting and harvesting can be utilized in some cases to remove rooted aquatic plants from the shores of reservoirs. In canals, mechanical removal by rakes and dredges is recommended.

On Volta Lake, Ghana, it has been found that villages located on beaches with no vegetation have a much lower prevalence of schistosomiasis than villages in protected coves with heavy vegetation. Wave action itself is detrimental to snail populations and it prevents establishment of permanent vegetation.

5. Proposed demonstration project 5; Physical measures, channelization of rivers and streams in transmission foci

High velocities and flood surges will dislodge snails from rivers or streams and this can be used to reduce snail population in local transmission foci. As it is expensive to channelize and pave natural rivers and streams in order to increase velocities, it is necessary to determine the precise extent of the local transmission zone. The channel improvements should then be concentrated in those areas where snail populations occur and shed cercariae into the transmission focus.

The velocity required to prevent snail colonization is about 30 centimeters per second in the immediate snail habitat. In deep streams, the cross sectional distribution of water velocity must be allowed for in calculating the required average stream velocity to ensure suffi-cient peripheral velocities adjacent to boundary surfaces.

Adequate increase in local velocities can often be obtained by manual clearance of weeds in streams and simple straightening and removal of obstructions and debris from the streambed.

In addition to reducing snail populations by increasing water velocity, channelization is a method of destroying snail habitats along the river edge.

6. Proposed demonstration project 6; Reduction of contamination of surface waters by protection of small reservoirs

The numerous small reservoirs found in arid zones are often sites of transmission. Therefore special attention should be given to ensure that these impoundments are as safe as possible.

Location

Impoundments should be located as far from housing or areas of human activity as is practicable and made inaccessible by fencing or hedges.

Reservoir use

When impoundments are used as water supply for people or cattle, the water should be drawn off by a pipe to a delivery point outside the fence or away from the reservoir.

Intakes

The intakes for water supplies should be located some distance away from shorelines and in the deeper sections of the reservoir.

Design considerations

Bank slopes should be as steep as practical to make human contact difficult, assist in vegetation control and decrease the shallow areas which are suitable for snail habitats.

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TABLE 4. Demonstration Project 4: Proposed Locations.

Country	Location	Type of Project	Local Contact
Brazil	Minas Gerais and Sergipe	Mechanical Weed Control	Dr. Ernani Mota S.U.C.A.M. Ministerio de Saude, Esplanada dos Ministerios, Blocco 11, Brasilia
Sudan	Gezira	Effects of present weed control by Ministry of Irrigation	Dr. A.H.S. Omer Institute of Tropical Medicine P.O. Box 1304, Khartoum

TABLE 5. Demonstration Project 6: Proposed Locations.

Country	Location	Type of Project	Local Contact
Nigeria	Plateau area	Modify existing ponds	Dr. E.A. Smith Federal Ministry of Health Lagos
Kenya	Coastal area, Machakos, and Lake Victoria	Construct barriers around existing and new ponds	Dr. T.K.A. Siongok Division of Vector Borne Diseases P.O. Box 20750, Nairobi

III. Gaps in present knowledge and recommendations for research projects

The Committee found a number of gaps in the present state of knowledge about environmental and habitat management methods for schistosomiasis control, including a need for assessment of costs and benefits of all techniques.

The following 10 research items of significance on both short and long-term basis were specified for consideration. The institutions listed after each item

were suggested as localities where, because of favourable local expertise and field situations, these specific topics may be effectively studied.

1. Research Proposal 1

a) *Investigate use of latrines and sanitation in preventing transmission*

No serious studies of the role of latrines in controlling *S. mansoni* transmission have been undertaken in over 20 years. The Committee's review of the few earlier studies indicate the specific effect

of provision of latrine facilities in reducing the rate of infection of people with *S. mansoni* to be inconclusive. It was appreciated that *S. haematobium* eggs may be spread to water and transmission remain high in spite of adequate provision of latrines. However, because of the obvious potential of effective sanitation for protection of snails from infection, this topic merits further investigation. Latrines should be installed in schools and at other public places to train children to use them. This measure should go with community health education within the context of local social, economic and cultural practices, and the provision of maintenance facilities. New designs for human waste disposal should be encouraged.

It is recommended that the investigations cover the general field of improving sanitary practice. The standard approach to health education, in sites where human and financial resources are limited, has not proved outstandingly successful in promoting the use of latrines. Greater understanding of how to change human behaviour in this respect is urgently needed and cooperation between UNEP and UNESCO's MAB environmental perception programme offers hope of progress in this difficult field.

b) *Suggested institutions*

— Institute of Public Health
Makerere University
Kampala, Uganda

— Volta Lake Research and Development Project
Akosombo, Ghana

2. **Research Proposal 2**
a) *Use of fish and other organisms in schistosomiasis control*

Many fish species have been found to feed on snails and cercariae and destroy aquatic vegetation, thus changing the snail habitat. Among these are African and South American cichlids and a North American sunfish. There are different freshwater puffer fishes whose favourite if not exclusive food is snails. Guppies which can also withstand polluted waters in which they are capable of attaining extremely high densities have been reported to feed on cercariae. Some species of the African genus *Tilapia* have a capacity for destroying aquatic vegetation and consuming algae with small snails and eggs. Examples have been given of *Tilapia* species in Zaire clearing artificial lakes of water weeds.

More study on the use of fish for controlling schistosomiasis is recommended, especially in small artificial water bodies where this approach appears more promising or at least easier to assess and control. The experiments should preferably be undertaken as side efforts of fisheries research or as part of interdisciplinary aquatic ecology research.

Similarly, additional effort is required for understanding better the potential of biological control in the context of schistosomiasis.

b) *Suggested institutions*

— Divisao da Peixes (Northeast Irrigation Systems) Departamento Nacional da Obras Contra Sequias and Ministry of Health of Federal Government, S.U.C.A.M.
Brasilia, Brazil

— Institute for Aquatic Biology
Center for Scientific and Industrial Research
Accra, Ghana

— Division of Vector Borne Diseases.
Ministry of Health
P.O. Box 20750
Nairobi, Kenya

— Kainji Lake Research Center
Nigeria

— East African Institute for Fisheries Research
Jinja, Uganda

— Liberian Institute for Biomedical Research
c/o Ministry of Health
Monrovia, Liberia

— c/o Prime Minister's Office, Teheran, Iran

  a) Centre for Endogenous Development Studies

  b) Department of the Environment

  c) Water and Power Agency, Dezful, Khuzestan

— Prof. A. Ceombes
Centre de Recherches de la Guadeloupe
Département de Biologie Animale
Centre Universitaire
Avenue de Villeneuve
6600 Perpignan, France

3. Research Proposal 3

a) *Use of plant materials as molluscicides*

Commercially available synthesized molluscicides are increasing in cost and their use limited by foreign exchange available in developing countries. A variety of plants growing in tropical countries have been known for some time to have molluscicidal properties. This information should be followed up. Although the «molluscicide» may not be as active as those formulations which are currently available, it may be more acceptable if, being «locally» made, it is cheaper.

It should not however be forgotten that, as with conventional molluscicides, aquatic biota other than intermediate

hosts of schistosomes are affected by the plant molluscicides and this and other long-term effects of their use should be investigated. The relative costs and benefits of these plant products should be compared with synthetic chemical molluscicides, including foreign exchange, development costs and ecological effects.

b) *Suggested institutions*

— Dr. W. Ahmed
Centre for Endogenous Development Studies
P.O. Box 938
Teheran, Iran

— Dr. A. Lemma
Institute of Pathology
P.O. Box 1176
Addis Ababa, Ethiopia

4. Research Proposal 4

a) *What characteristics in man-made lakes determine the presence or absence of intermediate snail hosts ?*

Since many reservoirs under design may eventually harbor intermediate host snails, it would be extremely valuable to be able to predict in advance which will be suitable habitats and to determine design modifications which could make them unsuitable. The critical factors to be investigated include water temperature, predominant vegetation which may be related to limiting nutrients or seasonal climatic variations, and the nature of the shoreline including beach slope and exposure to wave action.

b) *Suggested institutions*

— Institute of Tropical Medicine
P.O. Box 1034
Khartoum, Sudan

— Department of Zoology
University of Ibadan, Nigeria

and

— Federal Ministry of Health
Epidemiology Unit
Lagos, Nigeria

5. Research Proposal 5

a) *What modifications of irrigation techniques would be of direct benefit to schistosomiasis control ?*

Recent advances in irrigation practice may minimise the chances of creating snail habitats. It is desired that methods of assessment be evolved to evaluate these techniques on the basis of cost, applicability to land resources and local conditions and their effectiveness in endemic areas.

Co-ordination of the activities of agriculturalists, irrigation managers, health authorities and farmers will contribute to reduction of the spread of schistosomiasis. Investigations into institutional aspects of irrigation management will help to develop systems to minimise organisational friction, environmental damage, and disease transmission.

b) *Suggested institutions*

— Institute of Tropical Medicine
P.O. Box 1034
Khartoum, Sudan

— Dr. Solon Camargo
Conselho Nacional das Pesquisas &
S.U.C.A.M. (Caatinga do Moura)
c/o Ministerio da Saude
Explanada dos Ministerios
Blocco 11, Brazilia, D.F., Brazil

6. Research Proposal 6

a) *What are the minimum drawdown rates to be used in reservoirs for controlling snails ?*

In experiments in small reservoirs, it has been shown that periodic rapid drops in the water levels to cause stranding can be an effective method of controlling snail populations. However, the rate of drawdown requires a relatively large discharge of water and it should be determined if the use of lower recession rates and frequencies would also be effective. In addition, the rates required for all snail species should be determined.

Finally, improvement in design of automatic siphons to produce the required fluctuation patterns is needed to minimise wastage of water.

b) *Suggested institution*

— PRNC Division of Human Ecology
University of Puerto Rico
Caparra Heights Station
Puerto Rico 00935, U.S.A.

7. Research Proposal 7

a) *Use of native and associate snails as competitors and decoys in reducing transmission*

The objective is to demonstrate the efficacy of non-susceptible snails which occur in the same habitat with susceptible snails, in acting as decoys for consuming miracidia, and thus reducing transmission.

It has been demonstrated under laboratory conditions that several non-susceptible species of snails can act as decoys for miracidia. It is recommended that the potential of this phenomenon in reducing transmission be investigated.

It would be desirable to demonstrate the efficacy of native non-intermediate host snails as competitors against the snail hosts, since certain species of non-susceptible snails have been demonstrated to act as competitors against the snail intermediate hosts when introduced into

the same aquaria. This should be investigated in different geographic regions.

b) *Suggested institutions*

— Federal Ministry of Health
Epidemiological Unit
and

— Department of Zoology
University of Ibadan, Ibadan, Nigeria

8. Research Proposal 8

a) *Studies on competitive displacement among planorbid snails*

The objective would be to find out the possibility of the exclusion of intermediate snail hosts by the introduction into the same habitat of a species of the same genus resistant to infection.

By the old principle revised by de Bach, «different species which co-exist indefinitely in the same habitat must have different ecological niches». Observations made in Brasil indicate the possibility that competitive displacement involving *Biomphalaria* species has occurred in certain areas. Specimen of *B. glabrata* from the Northeast part of the country introduced into a breeding place in the State of Rio de Janeiro, excluded the autochthonous species from that habitat. The reverse occurred at Belo Horizonte, where the offspring of albino specimens of *B. tenagophila* from São Paulo introduced into a habitat replaced a population of the autochthonous *B. glabrata*.

Parallel to field studies on competitive displacement, laboratory and limited field experiments should be carried out to clarify several aspects of the competitive capability of the snail species involved.

b) *Suggested institution*

— School of Health Sciences
University of Brasilia

Brasilia, D.F. 70,000
Brazil

9. Research Proposal 9

a) *Threshold values of snail populations in limiting transmission*

One of the unsolved problems in the epidemiology and transmission of schistosomiasis is the size of the snail population in a certain habitat which is adequate to ensure transmission of the disease. Knowledge of this threshold value is significant in deciding on what degree of control is necessary in certain situations.

The emphasis given to different methods of schistosome control depends in part on the theoretical as well as practical justification for them. In this respect, further work on mathetical models of the environmental aspects of schistosome transmission and control are to be encouraged.

b) *Suggested institutions*

— Ross Institute of Tropical Hygiene
London School of Hygiene and Tropical Medicine
Keppel Street, Gower Street
London WCIE 7HT, England

— Department of Zoology
Ibadan, Nigeria

— WHO/UNDP Schistosomiasis Project
P.O. Box M 190
Accra, Ghana

10. Research Proposal 10

a) *Value of changing physical and chemical characteristics of water*

The physical and chemical conditions required for the laboratory growth and reproduction of aquatic snails are now reasonably well-known. Some factors

such as temperature and water quality are critical for reproduction and they warrant further investigations as methods of control in the field.

b) *Suggested institution*

— Department of Zoology
University of Ibadan
Ibadan, Nigeria

IV. Training

Training in environmental and ecological control methods should be supported for local field level technicians rather than for academic level personnel. For water supply systems this means engineering technicians, plumbers and maintenance personnel.

This is primarily a national and local matter but UNESCO should be encouraged to assist with setting up courses and training teachers for this group of technicians and artisans. At a higher level, it is equally necessary to broaden the attitudes of health professionals, and UNEP should support efforts to increase the environmental awareness of physicians and others specialising in public health for developing countries.

Training is also recommended for intermediate level managers who administer water resources programmes, and agricultural development agencies.

Mention is made of the 1-year course for high-level malacologists to be given in Rabat, Morocco, starting January 1976 by the Pasteur Institute and the Faculty of Medicine of Tunis. Attention is also drawn to the Institute of Tropical Medicine of Khartoum, Sudan, which has been selected for training schistosomiasis technicians and this should be given further support.

V. Implementation

Demonstration Programmes

Development of demonstration projects based on the principles outlined will depend on the request and involvement of countries. WHO and UNEP can only lend support as appropriate.

Since rice irrigation has become an important priority in the Sahel Zone and the International Bank for Reconstruction and Development is supporting projects on the Gorgol River in Mauritania, the Delta of the Senegal River in Senegal and the flood plain of the River Niger near Mopti in Mali, it is suggested that UNEP/WHO participate with the Bank in these projects. A supplementary ecological programme on Lake Volta might be added to the present WHO/UNDP programme for schistosomiasis control.

Research Items

A preference for funding of agencies and institutions in developing countries is recommended.

VI. Exchange of Information

It is recommended that a focal institution serve to establish and increase rapid exchange of information on the subject of ecological and habitat management methods for the control of schistosomiasis in co-operation with regional centers which would disseminate information to individual workers in each of the major endemic zones. A proposal has been made that the Edna McConnel Clark Foundation might consider being the focus for information collection and exchange.